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A literature review on the aquatic toxicology of petroleum oil: An overview of oil properties and effects to aquatic biota

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

This literature review gives an overview of petroleum oil:

1) Production, transport and historical spills in Canada,
2) Physical and chemical properties relevant to aquatic toxicity and,
3) Lethal and sublethal effects on fish and other aquatic biota.

Canada has large oil reserves. A significant proportion of total reserves are associated with Alberta's oil sands. Both conventional and unconventional oil production occurs in Canada and unconventional oil production is dominated by recovery of bitumen from oil sands deposits. Currently, total annual export of petroleum oil by pipeline is substantially greater than exports by marine and rail transportation systems. Historical spills of petroleum oil occurred from pipelines, rail and marine transport methods. Spills have been known to occur in both freshwater and marine ecosystems.

Physical properties and other environmental conditions (e.g. weathering, temperature, presence of sediments, etc.) influence the fate and behaviour of spilled oil in the environment. Chemical properties vary widely across the different classes of petroleum oil. In all oils, aromatic hydrocarbon components are associated with harmful effects in aquatic biota. Monoaromatic hydrocarbons, i.e., benzene, toluene, ethylbenzene and xylene, are often associated with acute toxicity. Meanwhile, polycyclic aromatic hydrocarbons can cause both acute and sublethal toxicity.

Several classes of harmful effects have been documented in the literature based on laboratory studies and from observations after oil spill accidents. Exposure to petroleum oil can cause several biological effects including increased mortality, early-life stage developmental defects, reduced reproductive capacity, genetic damage, impaired immune function and disease resistance, and changes in behaviour. Many publications reported that early-life stages (embryos and larvae) of fish are more sensitive to oil exposure than adults.

Several recommendations are made for future research to address knowledge gaps on the effects of oil on aquatic biota. Research should address the lack of information on the effects of oil sands products (e.g., diluted bitumen, synthetic crude oil) on aquatic biota. Research is also needed to study the fate, behaviour and effects on aquatic biota of oil spills in ice-covered Canadian waters.

RÉSUMÉ

La présente analyse documentaire donne un aperçu des produits pétroliers, c'est-à-dire :

1) leur production, leur transport et les cas de déversements de pétrole au Canada;
2) leurs propriétés physiques et chimiques qui sont pertinentes à la toxicité aquatique;
3) leurs effets létaux et sublétaux sur les poissons et autre biote aquatique.

Le Canada recèle d'importantes réserves pétrolières. Les sables bitumineux de l'Alberta représentent une proportion considérable des réserves totales. Le Canada produit du pétrole classique et du pétrole non classique, lequel provient en grande majorité de la récupération du bitume des gisements de sables bitumineux. À l'heure actuelle, le volume total des produits pétroliers exporté annuellement par pipeline est nettement supérieur au volume exporté par voies maritime et ferroviaire. Les pipelines, les trains et les navires ont tous contribué aux déversements accidentels d'huile de pétrole qui se produits. On a rapporté des déversements dans l'eau douce ainsi que dans les écosystèmes marins.

Les propriétés physiques et d'autres conditions environnementales (p. ex., l'altération, la température, la présence de sédiments) influent sur le devenir et le comportement du pétrole déversé dans l'environnement. Les propriétés chimiques varient grandement selon la classe d'huile de pétrole. Dans toutes les huiles, les hydrocarbures aromatiques sont associés à des effets nocifs sur le biote aquatique. Les hydrocarbures monoaromatiques (benzène, toluène, éthylbenzène, xylène) sont souvent associés à la toxicité aiguë. Les hydrocarbures aromatiques polycycliques peuvent donner lieu à une toxicité aiguë ou sublétale.

La documentation colligée à la suite d'études de laboratoire et d'observations faites après des déversements accidentels de pétrole fait état de plusieurs classes d'effets nocifs. L'exposition aux produits pétroliers peut provoquer plusieurs effets biologiques, dont une augmentation de la mortalité, des anomalies du développement au début du cycle de vie, une réduction de la capacité reproductive, des dommages génétiques, une altération de la fonction immunitaire et de la résistance aux maladies, et des changements de comportement. De nombreuses publications indiquent que les stades précoces de l'existence des poissons sont plus sensibles à l'exposition au pétrole que les stades adulte.

Plusieurs recommandations sont formulées pour entreprendre des recherches éventuelles qui combleraient les lacunes dans les connaissances actuelles sur les effets du pétrole sur le biote aquatique. Ces études devraient remédier au manque de données sur les effets des produits des sables bitumineux (p. ex., le bitume dilué, le pétrole brut synthétique) sur le biote aquatique. Il est également nécessaire d'étudier le devenir, le comportement et les effets des déversements de pétrole sur le biote aquatique dans les eaux canadiennes couvertes de glace.

1 INTRODUCTION

1.1 PURPOSE AND METHODOLOGY OF THE LITERATURE REVIEW

The purpose of this review is to provide an overview of the biological effects of petroleum oil on aquatic biota. The majority of the information presented pertains to finfish but also includes information on other aquatic biota such as shellfish and marine mammals. The review includes crude oils and petroleum products derived from crude oils but does not include natural gas and natural gas liquids or processed waters resulting from exploration and production activities. Throughout the review the term "oil" is used as a general term to describe a wide variety of petroleum oil.

This document is divided into four major sections. Section 1 defines the scope of the literature review and provides an overview of the extraction, processing and transportation of oil in Canada. In Section 2, aspects of the physical and chemical properties of petroleum oil are presented. In Section 3, the primary focus of this literature review, information on the acute and sublethal biological effects of oil on aquatic biota is summarized. In Section 4, research recommendations are listed. This section was developed as a result of discussions with participants at a Canadian Science Advisory Secretariat (CSAS) workshop for identifying research requirements for the biological effects of oil on aquatic ecosystems (DFO 2015).

The review presents research from real-life oil spills and laboratory experiments. Following several oil spill accidents, biomonitoring programs and research studies were implemented. These field studies are particularly valuable and complement research in laboratory settings, in part because they provide opportunities for observing effects at the population and community-levels, something that is not easily achievable in a laboratory. The main limitation of studies of real-life oil spill remains that associations between cause and effect are often difficult to make with certainty. In light of this, the review includes information from the whole spectrum of the available studies from controlled experiments to real-life accidental oil spills.

This literature review summarizes information from scientific journals and from government and industry reports and websites. The literature was queried using several search engines including Web of Science, Google Scholar, Google and the Department of Fisheries and Ocean's "Waves" library database.

1.2 BRIEF INTRODUCTION TO THE OIL INDUSTRY IN CANADA

1.2.1 Oil Production

Oil reserves in Canada are estimated at 27.5 billion m^3, of which 26.7 billion m^3 are found in Alberta's oil sand deposits and the remaining 0.8 billion m^3 are from conventional, offshore and tight oil formations (Natural Resources Canada (NRCan) 2014a). Conventional oil reserves on the East coast are estimated at 0.2 billion m^3 and Alberta's conventional and tight oil reserves are estimated at 0.3 billion m^3 (NRCan 2014a).

For 2014, Canadian production of crude oil and petroleum products is estimated at 598,208 m^3 per day (National Energy Board (NEB) 2014a). Alberta is estimated to produce 461,594 m^3 per day of petroleum liquids including crude oil, bitumen and condensate. Saskatchewan produced 82,497 m^3 per day and East coast offshore production is estimated at 36,584 m^3 per day (NEB 2014a).

The majority of the current and future production of crude oil is from three major deposits of bitumen; the Athabasca, the Cold Lake and the Peace River deposits (Canadian Association of Petroleum Producers (CAPP) 2014). These are all located in the province of Alberta and only the Cold Lake deposit is partially found in the neighboring province of Saskatchewan. For Eastern Canada, crude oil production accounts for an estimated 6% of Canada's total production and these were primarily from three offshore oil platforms (Hibernia, Terra Nova and White Rose) (CAPP 2014). In Canada's Arctic there is interest in exploring and extracting crude oil. Past exploration activities in the Arctic occurred in the 1970s and 1980s (BREA 2011).

Canadian oil production estimates for 2014 are summarized in Table 1. The average production of conventional light crude oil is estimated at 152,130 m^3 per day. Meanwhile, condensate and pentane production is estimated at 28,019 m^3 per day. Upgraded bitumen and non-upgraded bitumen, both produced in Alberta, represent 150,288 and 193,547 m^3 per day, respectively. Bitumen represents a major component of Canada's total production of petroleum products. Projections of future crude oil production estimate that total oil produced from oil sands sources will become much greater than that from conventional sources (CAPP 2014). This is as a result of substantial growth in the oil sands production compared to the slight increase in future production from conventional sources (CAPP 2014). Production of conventional crude oil is expected to continue at a fairly steady rate for the near future but some changes in extraction methods are likely in mature basins. In some areas, the application of horizontal multistage hydraulic fracturing will likely extend the life of some of these deposits and allow extraction to continue (CAPP 2014). Hydraulic fracturing techniques have also allowed access to tight oil reserves that were not previously extractable using conventional methods (NRCan 2014b). These tight oil reserves are generally associated with fine-grained impermeable rock units (NRCan 2014b).

Table 1. Canadian oil production estimates for 2014

Product type	**m^3/d**
Condensate/Pentane	28,019
Conventional light crude oil	152,130
Upgraded bitumen	150,288
Conventional heavy crude oil	74,225
Non-upgraded bitumen	193,547
Total	**598,208**

Source: Data adapted from NEB (2014a).

The majority of the crude oil extracted in Canada is exported to international markets for further refining. In 2013, 74% of the crude oil produced in Canada was exported to international refineries (CAPP 2014). In Canada, refineries are located in Atlantic Canada, Québec, Ontario, Saskatchewan and Alberta. In 2013, Canadian refineries processed 143,810 m^3 per day of western Canadian crude oil; 11,123 m^3 per day of crude oil produced in Eastern Canada and; 102,018 m^3 per day of foreign imports (CAPP 2014). There has also been some interest in expanding existing refineries and opening new facilities to increase refining capacity. In Eastern

Canada, proposed pipelines would increase the supply of crude oil from Western Canada sources and allow expansions of these refineries (CAPP 2014).

1.2.2 Processing of oil sands bitumen for transport

In general, crude oil derived from Alberta's oil sands is extracted using two different methods depending largely on the depth of the deposit relative to the surface. Oil sands deposits found near the surface are extracted by surface mining while deposits found deep underground are extracted by *in-situ* methods such as steam-assisted gravity drainage (SAGD). In 2013, total production of crude oil from oil sands by *in-situ* methods was 175,000 m^3 per day while the mining method produced 135,000 m^3 per day (CAPP 2014). Projections indicate that bitumen extracted by *in-situ* methods will substantially exceed surface mining production in future years (CAPP 2014).

In general, bitumen is upgraded to synthetic crude oil or diluted with lighter oils prior to transport. As a result of the high viscosity of natural bitumen, diluting is required to allow flow during transport by pipeline and for loading and unloading when using other transportation methods (e.g., rail cars). Currently, most bitumen from surface mining projects is upgraded to light crude oil (i.e., synthetic crude oil) while, bitumen from *in-situ* projects is generally not upgraded (CAPP 2014). The majority of the bitumen from *in-situ* projects is diluted with light hydrocarbons or with synthetic crude oil (CAPP 2014). The bitumen blended with synthetic crude oil is referred to as "SynBit" while, the bitumen blended with condensate is referred to as "DilBit" (CAPP 2014). The ratio of bitumen to diluent varies but SynBit is generally 50:50 and DilBit is generally 70:30. In some cases, bitumen transported by rail requires less diluent given some of the rail cars are equipped to heat the contents which decreases the viscosity of the bitumen (CAPP 2014). Diluents are usually natural gas condensate, naphtha or a mix of other light hydrocarbons. In addition, temperature influences the viscosity of bitumen. As a result, to maintain flow in transmission pipeline, the blending ratios of diluents to bitumen are modified seasonally to meet viscosity specifications (NRC 2013).

1.2.3 Transportation

In 2013, Canada's exports of crude oil amounted to 413,157 m^3 per day (CAPP 2014). The majority of crude oil export is by pipelines (NEB 2014b). In the past five years, the volume of crude oil exported by pipelines has increase substantially. Export of crude oil by rail has also increased substantially in recent years however; the volume remains small relative to volumes exported by pipelines (NEB 2014b). The volume of crude oil exported by marine transportation is also important and exceeds export volumes by rail transport. In 2013, total export of crude oil was over 130 million m^3 by pipeline; over 10 million m^3 by marine transport; and over 7 million m^3 by rail (NEB 2014b).

Currently, the network of pipelines is extensive in Canada. There are an estimated 825,000 kilometers of transmission, gathering and distribution lines used for natural gas and liquid petroleum in Canada (NRCan 2014c). Pipelines vary in diameter, and include 10-30 cm gathering lines; 1.3-15 cm local distribution lines and 10-122 cm large-diameter transmission lines (NRCan 2014c). There is a total of 105,000 kilometers of large-diameter transmission lines, and approximately half in operation have diameters greater than 45 cm (NRCan 2014c). There are also a number of proposed pipelines for increasing exports and imports of oil products. For example, part of the proposed Enbridge Gateway project is to construct twin pipelines, one for export of diluted bitumen and other oil products and one for import of condensates (CAPP 2014).

In most cases, pipelines do not carry a single type of petroleum oil. At any given time, a pipeline can transport several shipments of oil products as independent batches. These batches, as they

are termed in the pipeline transportation sector, are at least 8,000 m^3 in volume and cover a variety of crude oil grades (NRC 2013). In most cases, to reduce mixing between batches, the oil products are sequenced in a way to maximize difference in physical characteristics.

The rail network is also extensive in Canada (CAPP 2014). In recent years, oil has been increasingly transported using rail to meet demands of the market and to allow its transport to new markets that are not currently accessible to the distribution network of pipelines (CAPP 2014). In Canada, most of the rail system is operated by two large companies: Canadian Pacific and Canadian National Railways. For transport of bitumen by rail less diluent is needed as many of the rail cars are insulated or heated to maintain a lower viscosity. Also, bitumen destined for rail transport can have higher sulphur content relative to bitumen destined for pipeline transport. This is because regulations for transport by rail car allow for higher sulphur content in bitumen compared to regulations for transport by pipeline (CAPP 2014).

1.2.4 Spills related to transportation

Regulatory authority over transportation and distribution of crude oil and petroleum products within provinces resides primarily with provincial governments. The National Energy Board of Canada (NEB) is responsible for regulating interprovincial and international movement of oil in Canada. Incidents of spills of crude oil or petroleum products are investigated by the Transportation Safety Board of Canada (TSB) in releases occurring from federally-regulated pipelines, marine vessels and rail transport. Completed investigation reports are available on the TSB website.

The NEB regulates approximately 73,000 km of interprovincial and international pipelines (NEB 2014c). Spills of liquid hydrocarbons from NEB regulated pipelines averaged a total of approximately 141.1 m^3 per year between 2011 and 2013 (NEB 2014c). Spills occurred between 2 and 9 times per year from 2011 to 2013. Within Alberta, spills from non-federally regulated pipelines are reported to provincial authorities. Statistics for spill size and occurrence within the province of Alberta are prepared by the Alberta Energy Regulator (AER). In 2012, the length of AER-regulated pipelines was approximately 61,576 km for multiphase products (i.e., lines connected to oil wells and containing mixtures of oil, gas and water) and 20,272 km for crude oil (i.e., treated product from which initial processing has removed gas and water) (AER 2013). Another 36,161 km of pipelines are used to carry other hydrocarbons including high vapour pressure products such as natural gas liquids, butane, propane, ethane, ethylene, some condensates and low vapour pressure products such as fuel oil, gasoline, diesel, and some condensates (AER 2013). The majority of these pipelines are small diameter lines that measure 15 cm or less and carry products a short distance from oil fields to processing facilities. Large releases occur infrequently while small leaks and releases (i.e., <100 m^3) occur at a greater frequency, accounting for 96% of the total. Of the AER-regulated pipelines, accidental release of <100 m^3 of hydrocarbon liquids averaged 238 per year from 2008 to 2012 (AER 2013). In contrast, releases of hydrocarbon liquids from 100-1000 m^3 occur on average 2 times per year and those of 1000-10,000 m^3 occur 0.2 times per year from 2008 to 2012 (AER 2013).

Spills from tankers and other ships in Canadian waters are reported to the Canadian Coast Guard where they are recorded in the Marine Pollution Incident Reporting System (MPIRS) database. A recent report commissioned by Transport Canada used data from the MPIRS database to summarize the frequency of spills by size category from 2003 to 2012 (WSP 2014). No large spills of hydrocarbon liquids (>1000 m^3) were recorded during this time period. On average, the smallest category of spills (10-100 m^3), as identified in the report, occur 2.5 times per year (Fuel: 1.9 spills/yr; Refined cargo: 0.6 spills/yr; Crude: 0 spills/yr). Spills of 100 to 1000 m^3 occurred 0.7 times per year (Fuel: 0.6 spills/yr; Refined cargo: 0.1 spills/yr; Crude: 0 spills/yr). Historically, the largest spill of petroleum oil by a ship in Canadian waters occurred

on February 4, 1970 off the coast of Nova Scotia when the tanker Arrow spilled 200,000 m^3 of Bunker C fuel oil (Vandermeulen and Singh, 1994). In contrast, on the West coast of Canada, the largest spills experienced were much smaller. Historically, the two largest spills on the West Coast of Canada are the 1988 Nestucca oil barge spill (87.4 m^3 of Bunker C) (BC MOE 2014a) and the 2006 M/V Queen of the North ferry incident (225 m^3 of diesel fuel and smaller amounts of other hydrocarbon liquids) (BC MOE 2014b).

Rail accidents on federally-regulated railways, including those involving spills of petroleum oil, are investigated by the Transportation Safety Board of Canada. Most of the hydrocarbon liquid spills involved fuels such as diesel and gasoline, many of which were <20 m^3. A spill of heavy fuel oil occurred on August 3, 2005 when a train derailed near Lake Wabamun in Alberta and spilled 700 m^3 of Bunker C fuel oil, a portion of which ended up in the freshwater lake (Transportation Safety Board (TSB) 2005). Research relating to the fate and effects of the spill on aquatic biota was launched following this incident (Debruyn et al. 2007). Based on the TSB reports, only a few recent train incidents have involved spills of crude oil. Of the recent spills, a rail incident in July 2013 at Lac-Mégantic, Québec involved release of approximately 6,000 m^3 of crude oil (TSB 2014). An estimated 100 m^3 of the crude oil spilled entered Mégantic Lake and the Chaudière River (TSB 2014).

2 PHYSICAL AND CHEMICAL PROPERTIES OF OIL

This section reviews some of the key aspects of the chemical and physical properties of petroleum oil. There are many different types of petroleum oils and they have highly variable chemistry. Crude oil, for example, can be composed of complex mixtures of thousands of constituents. The relative proportion of these constituents is responsible for physical characteristics of oil and for their fate and behaviour when spilled into the environment. A full detailed analysis of the chemical composition of oil is beyond the scope of this report. Instead, select physical and chemical characteristics of oil are highlighted here as they relate to potential ecological impacts, i.e. physical aspects may regulate the behaviour of the product spilled in water and may ultimately determine exposure in biota. The chemical composition of the products is important to consider and toxic components are highlighted in this section of the report as well as in Section 3 (acute and sublethal effects). Finally, it is important to recognise that physical and chemical properties can be rapidly altered immediately following a spill into the environment. In some cases, this weathering of the oil can cause changes to the toxicity of the spilled product.

There are many resources reporting information on the physical and chemical properties of oil. Environment Canada has an oil properties database of several hundred oils that is publicly available online. Also, the oil and gas industry in Canada also provides data on select physical and chemical properties online (Crude monitor).

In general, physical properties that are often reported include American Petroleum Institute (API) gravity, density, sulphur content, water content, flash point, pour point, viscosity, surface and interfacial tension, adhesion, the equation for predicting evaporation, emulsion formation, and simulated boiling point distribution (Wang et al. 2003). The chemical composition of the oils can be reported by hydrocarbon groups, volatile organic compounds, *n*-alkane distribution, distribution of alkylated polyaromatic hydrocarbon (PAH) homologues and other priority PAHs as listed by the United States Environmental Protection Agency (US EPA) (Wang et al. 2003).

2.1 PHYSICAL PROPERTIES

Density is an important physical characteristic of oil and is generally used to categorise crude oil types as light, medium, heavy and extra heavy. The American Petroleum Institute (API) has

adopted the API gravity scale to classify the weight of oil. The API gravity is reported in degrees and is a measure of density relative to water. In freshwater, oils with values below 10° sink and above 10° float, whereas in seawater, oils with values below 6° sink and float above 6°. Most petroleum oils have API values above 10° (Table 2). In contrast, natural bitumen from Western Canada varies in density from 7° to 13° (NRC 2013). Natural bitumen, however, is normally blended with diluent prior to transport by pipeline or rail. Gas condensates often used as diluents are considerably less dense compared to other crude oil products and vary from 42° to 55° (Crosby et al. 2013). As a result, dilbit transported in pipelines from Alberta such as Cold Lake and Western Canadian Blend, have 5-year average API gravities of 18° to 19° (Crude Quality Inc. 2014).

Density is important in determining the buoyancy of the spilled substance in water. Although the initial fate and behaviour of petroleum hydrocarbon products can be determined by the density of the spilled substance, other factors are important such as mixing energy, presence of sediments and weathering processes. Most spilled oil will float at the water's surface. However, there are special circumstances where oil can sink in the water-column. For example, mixing and adherence of oil to heavier materials such as sediments can cause sinking in the water-column (Michel 2011). Mixing with sediments can occur when oil is near shore and when wave action creates oil-sediment aggregations. Oil-sediment aggregates can become neutrally buoyant or sink to the bottom. Evaporation of oil and the process of weathering can also increase the density of oil. The process of evaporation of the lighter constituents can contribute to increasing density and lead to sinking in some heavy oils (Michel 2011).

Oil in water can also partition into droplets or dissolved fractions. Viscosity is an important measure for estimating the rate of spreading and resistance to dispersing into droplets. Surface and interfacial tensions are measures important in determining droplet size (Government of Canada 2013). In a spill at sea, waves provide mixing energy that can break surface films of oil and distribute oil droplets into the water-column. This formation of oil droplets can also be facilitated by the application of chemical dispersants. Solubility of the oil determines the tendency for oil constituents to dissolve into water. Given that the dissolved fraction of oil can diffuse across biological membranes (e.g., gills), solubility is arguably the most important factor in determining toxicity to aquatic biota.

Little information is available on the fate and behaviour of diluted bitumen in water. Recent work by the Government of Canada (2013) was conducted to assess the properties and behaviour in a spill scenario of two diluted bitumen blends (Access Western and Cold Lake) produced in Western Canada. Experiments included laboratory-based testing in small vessels (2.2 L) and large outdoor wave tank tests. Similar to other conventional crude oils, diluted bitumen floated in seawater even after evaporation, exposure to light and mixing between tested temperatures of 0 to 15°C (Government of Canada 2013). When fine sediments were introduced with high-energy mixing in small vessels, the diluted bitumen mixture did sink or was dispersed as floating tarballs, in the highly-evaporated fractions. In contrast, in the wave tank study, the addition of fine sediments under conditions of breaking waves and current flow was not effective in dispersing the diluted bitumen (Government of Canada 2013). Documented accidental spills also give case-specific information on the possible fate and behaviour of spilled diluted bitumen. An example spill of diluted bitumen is the 2010 Kalamazoo oil spill. In July 2010, a pipeline rupture led to a spill of diluted bitumen into a tributary of the Kalamazoo River in Michigan, USA (Crosby et al. 2013). The US EPA reported that 10-20% of the spilled product sank to the bottom of the river (US EPA 2013). Samples collected from the river sediments showed that oil-sediments aggregates were present (Lee et al. 2012).

The fate and behaviour of other heavy petroleum products have been studied. In 2005, a train derailment led to a release of a heavy fuel oil and pole-treating oil into Wabamun Lake, Alberta

(TSB 2005). Research following the spill showed that a large proportion of the oil sank to the bottom and this was largely as a result of aggregation of the oil with sediments (Hollebone et al. 2011). Another product, Orimulsion, is an extra heavy oil that is an emulsion of 70% bitumen and 30% water and is stabilized with small percentage of proprietary detergent (<0.5%) (Svecevicius et al. 2003). The bitumen used to produce Orimulsion is generally from Venezuelan sources. The bitumen in Orimulsion has an API gravity that ranges in value from 7.8 to 9.3 (Tavel and Johnson 1999). Given this density range, Orimulsion is intermediate in density compared to freshwater and saltwater. In mesocosm experiments, Stout (1999) showed that Orimulsion generally sinks as particles in freshwater and can either sink or float in saltwater depending on the mixing energy applied.

Temperature also affects the buoyancy of conventional and non-conventional petroleum products in water. Short (2013) compared calculated densities of diluted bitumen and water as a function of temperature and salinity. Based on these estimates, the author suggested that some diluted bitumen could sink in brackish water and freshwater at low temperatures. Other experiments by the Government of Canada (2013) showed that heavy weathering of diluted bitumen in small vessels produced densities that exceeded that of freshwater at temperatures less than or equal to 15°C.

Persistence is another important characteristic of oil following a spill into the environment. Several characteristics of oil such as viscosity, adhesiveness, and evaporative character can influence its duration in the environment before degrading (Owens et al. 2008). The NRC (2003) categorized persistence of oil as the length of time oil remains in the environment following a spill. According to the generalized NRC's classification, gasoline and light distillates such as diesel persist for days; crude oils persist for months; and heavy distillates such as heavy fuel oil persist for years. Several environmental factors including temperature influence the rate of natural physical removal or weathering of oil (see Section 2.3). Some specific substrate conditions have also been found to extend persistence of some oils. For example, oil residues stranded on coarse-sediment beaches can sometimes persist for decades as factors such as sediment-oil adherence and oil penetration depth can reduce the influence of weathering processes (Owens et al. 2008). Other definitions of persistence are also used by various agencies and organizations to frame response requirements following a spill. In this context, an approach used in the U.S. is to define persistence of oil based on distillation temperatures and specific gravity of the product (US Oil Pollution Prevention Regulation 2014).

Table 2. Physical properties of petroleum product categories ordered by density

Physical properties									
Property	Units	Gasoline[a]	Diesel[a]	Light Crude[a]	Heavy Crude[a]	Diluted Bitumen[b]		Intermediate Fuel Oil[a]	Heavy Fuel Oil[a]
						Access Western Blend	Cold Lake Blend		
Viscosity	mPa·s at 15°C	0.5	2	5-50	50-50,000	347	285	1,000-15,000	10,000-50,000
Density	g/mL at 15°C	0.72	0.84	0.78-0.88	0.88-1.00	0.9253	0.9249	0.94-0.99	0.96-1.04
API gravity	°	65	35	30-50	10-30	20.9	21.0	10-20	5-15
Flash Point	°C	-35	45	-30 to 30	-30 to 60	<-5	<-5	80 to 100	>100
Pour point	°C	-	-35 to -1	-40 to 30	-40 to 30	<-25	<-25	-10 to 10	5 to 20
Interfacial tension	mN/m at 15°C	27	27	10-30	15-30	24.2	27.7	25-30	25-35

Sources: a: Range of values for petroleum product category. Modified from Fingas 2011a.
b: Values for two blends (not weathered) from Government of Canada 2013. Values presented here do not reflect range of possible values for diluted bitumen (range in values may exist due to differences in source, processing by different companies and changes in seasonal blends, i.e., winter vs. summer blends).

2.2 CHEMICAL PROPERTIES

Petroleum oil is primarily composed of hydrocarbons and this can represent up to 97% in some products but can be as low as 50% in heavy oils and bitumen (Speight 1999). Other chemical components include elements such as sulphur, nitrogen and oxygen and some metals such as nickel, vanadium and chromium. Hydrocarbons have generally been classified by their structure. Hydrocarbons can be grouped into saturates, aromatics, resins and asphaltenes. Saturates are hydrocarbons that contain the maximum number of hydrogen around each carbon and consists primarily of alkanes. Straight-chain saturate compounds from ≥C18 are often referred to as waxes. Olefins are unsaturated hydrocarbons that contain fewer hydrogen atoms and only exist in appreciable amounts in refined products. Aromatics have at least one benzene ring and are typically associated with toxicity in aquatic organisms. These include monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylene (referred collectively as BTEX) and also, polycyclic aromatic hydrocarbons (PAHs) (NRC 2003). PAHs comprise between 0 and 60% of the composition of oil (Fingas 2011a). In general, the lower molecular weight aromatic hydrocarbons are found in greater amounts in oil relative to those of higher molecular weight. One to three ring PAHs can account for up to 90% of the total aromatic hydrocarbons in oil while, four to six ring PAHs are found in lower concentrations (NRC 2003). Also, PAHs in oil are primarily alkylated and, non-alkylated congeners are found at low concentrations (NRC 2003). Resins and asphaltenes are polar compounds. Resins are smaller compounds than asphaltenes and are largely responsible for the adhesive qualities of oil (Fingas 2011a). Asphaltenes consists of large molecules and contributes significantly to the high viscosity and density of oil.

General chemical composition varies across crude oils and refined petroleum products formed from crude oil through a variety of distillation, cracking and blending processes. Table 3 summarizes hydrocarbon group composition and total trace metals for selected refined products (gasoline, diesel, intermediate fuel oil (IFO) and heavy fuel oil (HFO)) and crude oils (conventional light and heavy). In general, light and mid-range distillates (gasoline and diesel) and light crude oils contain a higher proportion of low molecular weight saturates and aromatics (BTEX and 2-ringed PAHs) than heavy crude oil, IFO and HFO. In contrast, heavy crude oil contains higher percentages of PAHs than light crude oil. Lighter petroleum products are characterized by their low proportion of waxes, resins and asphaltenes compared to the heavier crude oils and oil products. Trace metals can also be found in oil products and relative composition of metals differs between oil types. Total metal concentrations generally increase from light distillates and light crude oil to heavier oil products. Trace metals include aluminum, copper, nickel and vanadium (Meyer et al. 2007). Mercury is found in greater concentrations in light crude oil compared to heavy crude oil (Meyer et al. 2007).

Chemical characterization of petroleum often includes target analytes such as saturated hydrocarbons (includes *n*-alkanes from C_8 to C_{40}), select alkylated PAH homologues from C_1 to C_4 and US EPA priority parent PAHs. Composition is highly variable between and within oil types however there are some consistent generalities in hydrocarbon distribution.

Light and mid-distillate products are those in the C_3-C_{12} and C_6-C_{26} range, respectively. Normal alkanes (*n*-alkanes) are found in higher concentrations in light to mid-distillates compared to crude oils (Wang and Fingas 2003). In gasoline, BTEX is a major component of the aromatic hydrocarbon composition (Table 3). The distribution of alkylated PAHs in products such as gasoline and diesel are dominated by the naphthalene series of PAHs (Wang and Fingas 2003).

For conventional (light) crude oil, the relative proportion of total PAHs is greater compared to the light and mid-distillate products. In crude oils, the naphthalene series of PAHs are found in greater concentration relative to other target PAH analytes (Wang et al. 1999). Typical

distribution profiles of alkylated PAH compounds in conventional crude oil follows C_0- < C_1- < C_2- ≈ C_3- > C_4- (Yang et al. 2011). In heavy crude oil and fuel oils (intermediate and heavy), PAH distribution shifts towards higher proportions of both naphthalene and phenanthrene series (Wang et al. 2003). Conventional crude oils and fuel oils are generally dominated by two and three ringed aromatic hydrocarbons (Wang et al. 2003). In comparison to crude oils, heavy fuel oils have lower concentrations of BTEX, higher concentrations of high molecular weight alkanes and higher concentrations of alkylated PAHs (Wang et al. 2003).

In the oil sands products, diluted bitumen and synthetic crude oil (upgraded bitumen), the concentration of total PAH is much lower compared to other crude oils and the relative proportion of the naphthalene series is lower (Yang et al. 2011). In comparison to conventional crude oils, oil sands products are composed of proportionally greater amounts of 3- to 5-ringed alkylated PAH (Yang et al. 2011). The distribution within PAH families differs compared to crude oils showing an increasing profile following C_0- < C_1- < C_2- < C_3- < C_4- (Yang et al. 2011; Government of Canada 2013). In addition, an important feature of the hydrocarbon profile of oil sand products is the significant presence of unresolved complex mixtures (UCMs) (Yang et al. 2011). UCMs are generally most pronounced in weathered and biodegraded oils (Melbye et al. 2009).

The low molecular weight aromatic hydrocarbons are highly toxic to aquatic organisms. These include monoaromatics and 2-ringed PAHs. These components are generally highly soluble, and thus acutely toxic. These low molecular weight constituents are also highly volatile compounds and generally remain in the environment for hours to days following a spill. In contrast, higher molecular weight constituents such as PAHs with 3 or more benzene rings are less soluble but are also less volatile and remain longer in the environment. Many studies have associated 3- to 5-ringed PAHs to chronic effects in fish, especially in early-life stages of fish (Carls et al. 1999; Hodson et al. 2007; Adams et al. 2014; Bornstein et al. 2014). In heavy fuel oil, an effects-driven chemical fractionation study demonstrated that fractions containing 3-ring to 5-ring PAHs and alkyl-PAHs were the most toxic to rainbow trout (*Oncorhynchus mykiss*) embryos (Bornstein et al. 2014). Meanwhile, fractions containing waxes and long-chain alkanes showed lower toxicity (Bornstein et al. 2014).

Alkylated PAHs are generally more abundant than their un-substituted counterparts in petroleum products and can be more toxic. Substituted PAHs, including alkylated and hydroxylated derivatives, are more abundant and can persist longer in the environment than un-substituted PAHs (Barron and Holder 2003). Alkylated substitution increases molecular weight, increases lipophilicity, reduces volatility, and decreases hydrophilicity. Additionally, some alkyl-PAHs (e.g., 7-isopropyl-1-methylphenanthrene; Retene), can be up to ten times more toxic than their un-substituted congener (Turcotte et al. 2011). While typically grouped to simplify complex environmental scenarios, many individual PAHs exhibit differential toxicity to organisms.

Table 3. Chemical properties of petroleum product categories ordered by density

Chemical properties								
Group	**Compound Class**	**Units**	**Gasoline[a]**	**Diesel[a]**	**Light Crude[a]**	**Heavy Crude[a]**	**Intermediate Fuel Oil[a]**	**Heavy Fuel Oil[a]**
Saturates	total	%wt	50-60	65-95	55-90	25-80	25-35	20-30
Olefins	total	%wt	5-10	0-10				
Aromatics	total	%wt	25-40	5-25	10-35	15-40	40-60	30-50
	BTEX	%wt	15-25	0.5-2.0	0.1-2.5	0.01-2.0	0.05-1.0	0.00-1.0
	PAHs	%wt		0-5	10-35	15-40	30-50	30-50
Polar compounds	total	%wt		0-2	1-15	5-40	15-25	10-30
	resins	%wt		0-2	0-10	2-25	10-15	10-20
	asphaltenes	%wt			0-10	0-20	5-10	5-20
Metals	total	ppm	0.02	0.1-0.5	0-2	0-5	0.5-2.0	2-4

Sources: a: Range of values for petroleum product category. Modified from Fingas 2011a.

2.3 ENVIRONMENTAL FACTORS THAT AFFECT CHEMICAL AND PHYSICAL PROPERTIES

There are several factors that can affect the fate and behaviour of petroleum oil following a spill into the aquatic environment. These changes in fate and behaviour are important considerations for potential exposure and toxicity to aquatic biota.

2.3.1 Weathering

Following a spill into the environment, petroleum oil and products begin to undergo weathering. Weathering processes include spreading, evaporation, biodegradation, emulsification, oxidation, dissolution into water and sedimentation. The rate of weathering will depend on the environmental factors such as the temperatures, light conditions and wind mixing energy. Biodegradation by bacteria also contributes to degradation of hydrocarbons. Environmental conditions such as temperature and oxygen levels and the number of benzene rings in PAH molecules can affect biodegradation rates (Wang et al. 1998). Immediately following a spill, evaporation is an important weathering process. In the first few days, the volume of light crude oils can be reduced by up to 75% and medium crude oils up to 40%. In contrast, heavy oils lose about 5% of their volume during the first few days following a spill (Fingas 1999).

During the weathering process, chemical composition of petroleum oil changes substantially. Low molecular weight *n*-alkanes are depleted and the proportion of high molecular weight *n*-alkanes increases (Wang et al. 2005). For the aromatics, the BTEX group and C_3-benzenes become depleted and eventually are completely lost (Wang et al. 2005). The 2-ringed naphthalene PAH series decreases substantially relative to the other alkylated PAHs (Wang and Fingas 2003). As a result, the hydrocarbon composition of weathered oil shifts towards a higher concentration of high molecular weight aromatics compare to fresh oil. The remaining high molecular weight components including PAHs with greater than 3 rings, waxes, resins and asphaltenes are more resistant to weathering processes and persist longer in the environment (NRC 2003). The process of weathering also causes an increase in viscosity and density of the remaining mixture and this can contribute to changes in distribution in the water-column (Wang et al. 2005).

2.3.2 Salinity

Salinity can affect the solubility of oil components. For alkanes and aromatic hydrocarbons, solubility in water decreases with increasing salinity (Xie et al. 1997). For biota, this increased solubility in freshwater relative to saltwater corresponds to an increase in bioavailability of potentially toxic components of oil. In laboratory experiments with euryhaline fish (i.e., capable of adapting to a wide range of salinities), a decrease in salinity from seawater to freshwater produced increased solubility and uptake of PAHs by fish (Ramachandran et al. 2006). This increased solubility in freshwater was found to be more pronounced for low molecular weight 2- and 3-ringed PAH homologs compared to higher molecular weight 4-ringed PAHs such as pyrene (Ramachandran et al. 2006). This could have important implications when considering bioavailability of oil components in saltwater, estuarine and freshwater. Also, in spills that occur in marine systems, drifting oil into coastal and estuarine areas could make oil progressively more bioavailable to organisms as these waters are typically less saline.

2.3.3 Cold water environments

Special conditions such as cold temperatures and the seasonal or permanent presence of sea-ice can affect weathering dynamics of spilled oil and potentially, exposure of oil components to aquatic organisms. In the early 1980s, a small experimental spill of medium crude oil was conducted near shore of the northern end of Baffin Island in Canada's Arctic (Sergy and Blackall

1987). A large fraction of the oil became stranded on shore and weathering over the next two years was dominated by physical processes (Sergy and Blackall 1987). Twenty years later, researchers revisited the site to study the long-term fate of a spill in the Arctic and found variance in the level of degradation of the oil since the original spill (Prince et al. 2002). They found that in some samples the stranded oil was relatively unaltered while in others there was up to 87% loss of total hydrocarbons. Prince et al. (2002) concluded that biodegradation was a major factor in the fate of the spilled oil and hypothesized that the limitation for biodegradation at some sites was related to the lack of nutrients for bacterial growth.

The fate and behaviour of oil on the sea surface can also be affected by environmental conditions of northern latitudes. In Norway, a series of experimental spills showed that weathering of oil on the sea surface can depend on the presence of ice and its effects on decreasing wave energy (Brandvik and Faksness 2009). Brandvik and Faksness (2009) showed that total evaporative losses and water-in-oil emulsification of a light crude oil were substantially greater in open-water conditions compared to high ice coverage conditions (90% coverage).

2.4 OFFSHORE SPILL RESPONSE

2.4.1 Chemical dispersants

Several countermeasure techniques are available to responders following a spill at sea. Chemical dispersants have been used as an agent to aid in the dispersal of oil into the water-column. This section is a brief review of chemical dispersant properties. More complete summaries of dispersant properties are available in the literature (NRC 2005; Fingas 2011b); aspects of efficacy of dispersants are not discussed in this report.

Dispersants are used for the remediation of oil spills with the intention of preventing or reducing the impact of crude oil in aquatic ecosystems. While dispersants do not reduce the amount of oil entering the environment, they directly affect the fate, transport, and potential effects of an oil spill by altering its physical properties (NRC 2005). Dispersants generally consist of anionic and non-ionic surfactants, which act to break up the oil slick by promoting the formation of small oil-surfactant micelles (George-Ares and Clark 2000). These stabilized oil droplets disperse into the water-column, thus reducing the amount of oil floating as slicks. Authorities have used dispersants primarily to reduce the risk of oiling of shorelines but also to promote biodegradation and to reduce the risk of oil contact for biota that frequent the sea surface (NRC 2005). Studies have also shown that the application of chemical dispersants enhances partitioning of PAHs into the water-column in comparison to physical dispersal alone and thus potentially increases bioavailability and exposure to pelagic species (Lee et al. 2008).

Formulations of chemical dispersants have changed over the years. Laboratory tests show that dispersant formulations used in the 2000s are less acutely toxic to fish than those used in the 1970s (Fingas 2011b). The potential toxicity of current formulations of dispersants and their effects on the bioavailability of oil is discussed further in Section 3.6 of this report.

In general, chemical dispersants contain a high percentage of one or more uncharged or charged anionic surfactants of different solubility (Hemmer et al. 2011). Surfactants are amphiphilic molecules that exhibit both hydrophilic and hydrophobic properties and cause a decrease in the tension between water and oil. This leads to the formation of small oil-surfactant micelles less than 100 µm (Hemmer et al. 2011). Compared to physical dispersion alone, the size distribution of oil droplets is generally smaller when chemically dispersed (Li et al. 2008). This increases the ratio of surface area to volume of the droplets and promotes physical and biological degradation processes. Most dispersants also include a solvent such as petroleum distillates in their formulation to increase dissolution of surfactants (Hemmer et al. 2011).

2.4.2 *In-situ* burning

Under some conditions, *in-situ* burning may be used to remove oil floating on the sea surface. Research has been conducted to determine if burning changes the chemical composition of the water-soluble fraction of oil. In a laboratory experiment, crude oil surface slicks were created in small mesocosms (Faksness et al. 2012). In manipulated treatments, the surface oil slicks were burned while the reference treatments were left unburned. The experiment showed that chemical composition of the water-soluble fraction before and after burning was essentially unaltered. Acute toxicity tests with copepods showed no difference between treatments (Faksness et al. 2012).

3 TOXICITY OF CONTAMINANTS IN OIL

3.1 CONCEPTS OF TOXICITY

Toxicity can be defined as the negative effects on organisms caused by exposure to a chemical or substance. These negative effects may be lethal or sublethal. The concept of bioavailability of a substance is also important in determining toxicity to organisms. Toxicity can occur when bioavailable substances are absorbed by the organism and interact with sites of action to cause harm. Both chemical and physical characteristics of a substance in the environment are determinants of the availability of a substance to organisms. Exposure concentrations and the duration of exposure are also important determinants of toxicity. Exposure can be acute (i.e., short period) or chronic (i.e., prolonged period). The exposure period considered for acute and chronic exposures is relative to the life span of the species studied. Typical exposure periods for measuring acute toxicity in laboratory settings for fish and crustaceans are 24, 48 and 96 h. A common approach for acute toxicity testing is the LC50 test; which determines the concentration at which exposure to a substance causes mortality to half of the test population under standard laboratory conditions.

Sublethal endpoints are also important measures of toxicity. Some examples of sublethal effects that may be measured are: external and internal lesions, developmental abnormalities in early-life stages and abnormal molecular-level activity or behavioural changes in feeding and breeding. In some instances, toxic effects can eventually lead to death of the individual but, in many cases, sublethal effects cause change in populations without directly causing mortality of individuals. Delayed effects are also a possibility where an organism can be exposed to a substance acutely and, after a period of time, begin to exhibit measurable effects. It is possible that long-term exposure can lead to effects that only become observed later in life or in subsequent generations.

In addition, bioaccumulation, in combination with toxicity data, can be used in hazard assessments of substances. Bioaccumulation can occur when species experience long-term exposure and have a low rate of excretion of the substance relative to uptake. As a result, substances can accumulate in the organism over a period of time and can, in some instances, cause harm. Tissue concentrations of the substance of concern are often included for assessment of bioaccumulation.

3.2 METHODOLOGY OF OIL TOXICITY STUDIES

3.2.1 Laboratory studies

Several approaches to laboratory studies are possible depending on research objectives. In many cases, an objective of laboratory studies has been to characterize the toxicity of petroleum products relative to others using a standard suite of aquatic organisms. In this case,

standardized approaches such as those defined by the Chemical Response to Oil Spills: Ecological Research Forum (CROSERF) (Singer et al. 2000) are often used to determine lethal concentrations. Other research objectives have worked towards characterizing a more complete understanding of a wide range of chronic effects, identifying sensitive species and life stages and for developing an understanding of the mechanisms of toxic action. Overall, laboratory studies have allowed researchers to have greater control over environmental variables compared to field-based experiments.

To address issues of standardization when characterizing the toxicity of oil, CROSERF experimental and analytical protocols were developed to allow for comparison between laboratories but also to address some methodological problems that were identified related to the complex nature of crude oil as a toxic substance in aqueous solution (Singer et al. 2000). One of the major sources of inconsistencies identified between studies was attributed to the frequent use of nominal loading rates of oil and dispersants rather than using actual measures of the dissolved substances (e.g., Adams et al. 1999). The primary reason for the establishment of these standards was to improve protocols to evaluate the relative toxicity of dispersed versus non-dispersed oil. Because dispersants alter the amount of oil dissolved in the water it was argued by researchers that actual measures were critical to determine hydrocarbon concentration (NRC 2005). CROSERF toxicity test methods and protocols have been published in various scientific and technical publications (e.g., Singer et al. 2000; Aurand and Coelho 2005). Some authors have made modifications to existing CROSERF protocols to better suit research questions and applicability of results. For example, recommendations have been made to include analytical chemistry of a suite of PAHs, including alkylated homologs, rather than limiting analyses to total petroleum hydrocarbons (TPH) and volatile organic compounds (VOC) (Barron and Ka'aihue 2003). This was recommended as it has been shown that PAHs are important determinants of toxicity. Some authors further argue that tissue concentrations of hydrocarbons should be analyzed to provide measure of bioaccumulation but also to better associate exposure to observed effects (Barron and Ka'aihue 2003). Finally, researchers have included measures of sublethal endpoints in addition to mortality, which has significantly contributed to a more complete understanding of effects and mechanisms of oil toxicity on aquatic species.

Laboratory studies in oil toxicity have been shown to be complementary to research conducted in the field. Two large mesocosm installations in the US have been designed to study aspects of marine oil spills in both pelagic and near-shore environments (Reilly 1999). In a review of these installations, Reilly (1999) recommended that these types of mesocosm systems should be used in combination with a laboratory research program to enhance experimental precision. In some cases, laboratory experiments have been shown to successfully predict and model effects of those observed in actual oil spills into the environment (Carls et al. 2002).

3.2.2 Field studies

In general, field studies allow for less control of environmental variables but allow for investigations that may not be possible in most laboratory experiments (e.g., multi-species assemblages). Field experiments are also more costly and regulatory approvals from various stakeholders are sometimes difficult to obtain. Some examples of field studies on oil toxicity have included work with mesocosms (Siron et al. 1993; Powers et al. 2013), controlled spills into the environment (Prince et al. 2002) and opportunistic research following accidental spills. In general, most field research involving accidental spills are undertaken following large environmental spills (e.g., Exxon-Valdez tanker spill, Deepwater Horizon spill). Opportunistic research studies on smaller-scale spills could also improve understanding of impacts to aquatic environments.

In the literature, there are a substantial number of published studies on the impacts of large oil spills on biota. In North America, two examples of major accidental oil spills are: the Exxon-Valdez oil spill in Alaska in 1989 and the Deepwater Horizon oil spill in the Gulf of Mexico in 2010. Several studies were conducted by government agencies and academia on the fate and the effects of these spills. A bibliography of research publications for the Deepwater Horizon oil spill is published by the U.S. National Oceanic and Atmospheric Administration (NOAA) (NOAA 2014a). In general, these field studies benefit from a high degree of environmental realism but challenges exist where pre-spill data is lacking. Baseline studies have been beneficial in separating the influences of environmental variability and oil spill impacts.

3.3 EXPOSURE PATHWAYS

Diffusion of dissolved hydrocarbons across membranes is an important exposure pathway for aquatic biota. Bioavailability of dissolved hydrocarbons is generally greater compared to those bound to particles (NRC 2003). For example, experiments using blue mussels (*Mytilus edulis*) showed that accumulation of PAHs in tissues was best explained by dissolved phase concentrations rather than particulate phase concentrations (Pruell et al. 1986). In general, soluble components of oil can diffuse through gills, skin and other membranes of organisms. In fish, gills are an important membrane for diffusion given their role in gas exchange and their relatively large surface area (NRC 2003).

Biota interactions with hydrocarbons bound to suspended particulates and sediments can also be an important pathway of exposure (e.g., by contact or by ingestion). Hydrophobic PAHs can adsorb to suspended particulates and bottom sediments. PAHs associated with these sediments have been shown to be available to benthic biota, especially invertebrates (CCME 1999), but also benthic fish and deposited fish eggs. Benthic biota can take up PAHs bound to sediments through their diet, or by direct contact. Some benthic invertebrates have also been shown to bioaccumulate PAHs however, there is also evidence that some species can metabolize PAHs (Meador et al. 1995). In laboratory experiments, Meador et al. (1995) demonstrated that an accumulation of high molecular weight hydrocarbons (>3 ringed PAHs) in polychaetes was due to direct consumption of PAH-bound sediments while accumulation of low molecular weight PAHs (2- and 3-ringed PAHs) in both polychaetes and amphipods were due to diffusion of PAHs from interstitial waters of sediments.

In addition, filter-feeding bivalves can be exposed to oil constituents via diet by consuming suspended oil droplets or oil-bound particulates. When spilled into water, oil can partition into dissolved and oil droplet forms. Some oil droplets that form are small enough to be ingested by filter-feeding bivalves. For example, Payne and Driskell (2003) showed that filter-feeding mussels and oysters were especially susceptible to this route of exposure. Following an oil spill off the coast of Oregon, USA, these organisms generally had elevated levels of intermediate molecular weight hydrocarbons in their tissues correlating with the chemical composition of oil droplets, suggesting direct consumption of oil droplets and/or sediment-bound hydrocarbons. Meanwhile, tissues of dungeness crabs (*Metacarcinus magister*) were primarily contaminated with lower molecular weight hydrocarbons, similar to the chemical profile of the dissolved oil fraction (Payne and Driskell 2003).

Environmental availability of oil in water is influenced by factors such as hydrophobicity of oil constituents but also by other factors such as temperature, salinity, mixing energy, presence of inorganic particulates, the use of dispersants and the degree of weathering. Hydrophobicity is the tendency of a chemical to repel water and affects its interaction with environmental compartments (e.g., water, sediments, and suspended particles). A relative measure of hydrophobicity is the octanol-water partition coefficient (K_{OW}). The K_{OW} value is measured as the equilibrium ratio of the concentration of a chemical in *n*-octanol to its concentration in water.

Chemicals with low K_{OW} values (e.g., <5) are more likely to be present in dissolved form in the water-column and thus, potentially available to biota by transfer through membranes (Erickson et al. 2008). In contrast, chemicals with higher K_{OW} values have a stronger affinity for organic materials such as suspended particles and sediments.

Molecular weight of hydrocarbon molecules is also an important factor determining diffusion from environmental compartments to biota. Overall, PAH solubility decreases as molecular weight increases and higher molecular weight PAHs tend to bind more strongly to particulates and sediments (CCME 1999). Lower molecular weight compounds such as monoaromatic hydrocarbons and naphthalene are highly soluble and readily diffuse through membranes (French-McCay 2004). However, these lighter compounds are also very volatile and do not tend to persist in the environment for more than hours to days. In contrast, PAHs with three or more rings are more hydrophobic and thus, less soluble compared to low molecular weight hydrocarbons (French-McCay 2004). However, due to their increased persistence after a spill, these PAHs can be important components causing toxicity.

3.4 LETHAL TOXICITY OF PETROLEUM OIL

A gradient from lethal to sublethal effects can occur as a result of exposure to hydrocarbons. Mortality of aquatic species can occur after short-term exposure following an oil spill into the environment. For several species, laboratory studies have been undertaken to better characterize the range of concentrations of hydrocarbon components that can lead to mortality. This section briefly reviews: field observations of mortality following environmental spills, concentrations of hydrocarbons that can cause mortality, and the mechanism of action often associated with mortality (i.e. narcosis).

3.4.1 Invertebrates

Several studies have shown zooplankton to be susceptible to oil exposure. A study of coastal and oceanic zooplankton exposed to the water-accommodated fraction of fuel oil suggested that mortality was dependent on exposure time rather than exposure concentrations (Lee and Nicol 1977). It might also be possible for some copepod species to sense the presence of oil in water and, in some situations, actively avoid these areas (Abbriano et al. 2011). Exposure of *Calanus finmarchicus*, a common marine copepod species and an important prey for baleen whales, to crude oil showed relatively low LC50 (96 h) values ranging from 0.7 – 1.0 mg THC (total hydrocarbon content)/L (Hansen et al. 2012).

Gulf mysid shrimp (*Americamysis bahia*) are commonly used in toxicity testing in the USA. LC50 values (96 h) of larval mysids exposed to physically dispersed oil has been measured in the range of 0.15 to 83.1 mg TPH (total petroleum hydrocarbons) /L (as reviewed in NRC 2005). In contrast, the sensitivity of other crustacean species to crude oil has not been studied extensively. For example, there are few publications reporting LC50 values for American lobster (*Homarus americanus*), an important commercial species in Atlantic Canada. LC50 values (96 h) reported for early-life stages (first-, third- and fourth-stage larvae) of American lobster were between 0.86 to 4.9 mg TPH/L (Wells and Sprague 1976).

Some benthic invertebrates such as bivalves are especially sensitive to crude oil as they are generally less mobile and cannot avoid contaminated waters. Most bivalves are also filter-feeders and can consume oil droplets and oil-bound particulates that become suspended in the water-column. LC50 values (96 h) reported for early-life stages of molluscs are in the range of 1.14 – 1.83 mg TPH/L (as reviewed in NRC 2005).

3.4.2 Finfish

Evidence to date suggests that embryo and larval life stages of fish are more sensitive to oil toxicity than adults (NRC 2005). It is possible that the smaller size, less developed metabolic capability and differences in cell permeability of early-life stage organisms contributes to their enhanced sensitivity (Georges-Ares and Clark 2000). In general, early-life stages are also less mobile and unable to actively avoid areas where spills occur. In addition, some groundfish species have early-life stages that are pelagic and can be exposed to oil if present near the surface or in the water-column.

A wide range of concentrations of hydrocarbons have been found to cause mortality in fish. The range in reported values may be due to species-specific sensitivities but also other factors such as differences in experimental methods, analytical capabilities and type of petroleum product tested. Very low concentrations of aqueous PAHs (ppb range) have been shown to cause mortality to early-life stages of fish. For example, low ppb range of TPAH (0.7-7.6 ppb) caused mortality when Pacific herring (*Clupea pallasi*) eggs were exposed to weathered Alaska North Slope crude oil (Carls et al. 1999). Direct comparisons of species sensitivities to PAHs are difficult to make and should be interpreted with caution as toxicities largely depend on chemical composition of the oil tested. Some studies have attempted to compare lethal toxicity of oil across several species using identical methodologies. In a study comparing the sensitivity of adults and juveniles of nine Alaskan fish species exposed to static dilutions of water-soluble fractions of Cook Inlet crude oil and No. 2 fuel oil, LC50 (96 h) values ranged from 0.15 to >12 mg/L of total aromatic hydrocarbons (Rice et al. 1979). The National Research Council (2005) reviewed publications reporting LC50s for fish exposed to physically dispersed oil. This review reported LC50 values (from 24 to 96 h tests) for larval fish mortality exposed to water-accommodated fractions of oil to range from 0.045 to 40.20 mg TPH/L (NRC 2005).

Oil spills into the environment can cause extensive fish mortalities. The extent of fish mortality can depend on a number of factors but the size and volume of the receiving environment are likely important in determining exposure. In particular, extensive fish mortalities have been reported for oil spills into small freshwater rivers. In one example, in 1979, a pipeline carrying crude oil ruptured spilling approximately 1500 m^3 of oil into Asher creek in Missouri. This led to the mortality of an estimated 42,000 fish along an 8 km section of the creek (Crunkilton and Duchrow 1990). More recently, in 2000, a pipeline rupture caused significant fish mortality in Pine River, BC. Approximately 476 m^3 of crude oil was released into Pine River causing mortality to an estimated 15,000 to 250,000 fish (reviewed in Goldberg 2011).

3.4.3 Sea turtles

Exposure to hydrocarbons can also cause mortality in reptiles. Inhalation of toxic fumes may be an important route of exposure for sea turtles when they breach the surface to breath air (NOAA 2010). Additionally, sea turtles nest on shore and thus, females, eggs and hatchlings can be exposed when in contact with contaminated sediments (NOAA 2010). There is also evidence that sea turtles can ingest oil incidentally and that they do not seem to have the ability to avoid spilled oil (Lutcavage et al. 1995). In the Gulf of Mexico, during and following the 2010 Deepwater Horizon oil spill, an increase in sea turtle mortality was recorded (NOAA 2013). The cause of death is being investigated, but it is possible that exposure to oil is a contributing factor.

3.4.4 Marine mammals

Following an oil spill, the acute toxic effects to marine mammals can include those caused by direct contact with oil. Direct contact can cause fouling in fur-bearing marine mammals such as otters and seals. This can cause thermal stress in these species as oiling of fur can increase

thermal conductance and thus reduce thermoregulation abilities (Kooyman et al. 1977). Following the Exxon-Valdez oil spill, sea otters (*Enhydra lutris*) were particularly affected. Mortality estimates were in the hundreds for the Prince Williams Sound population (Garshelis and Johnson 2013). Mortality of many heavily and moderately oiled sea otters in the days following the spill was associated with hypothermia, likely a result of loss of insulation from oiled fur (Lipscomb et al. 1994). In an experiment with another fur-bearing marine mammal, ringed seal (*Phoca hispida*), it was found that short term exposure (24 hours) to crude oil did not cause mortality when returned to uncontaminated waters (Geraci and Smith 1976). It was noted however, that the short-term exposure did cause sublethal effects including transient eye damage, kidney and possibly liver lesions (Geraci and Smith 1976).

Marine mammals are also susceptible to surface oil slicks as they must come to the surface to breath, potentially becoming exposed by inhalation to volatilizing compounds. Immediately following the Exxon-Valdez oil spill, researchers determined that an estimated 302 harbour seals (*Phoca vitulina*) in Prince William Sound, Alaska died and it was suggested the cause of death was primarily due to inhalation of toxic fumes leading to brain lesions, stress, and disorientation (reviewed in Peterson 2001). Necropsies of sea otters suggested that inhalation of vapors could have led to the occurrence of interstitial pulmonary emphysema and contributed to sea otter mortality, particularly for those that were not heavily oiled (Lipscomb et al. 1994). It is possible that cetaceans in Prince William Sound also experienced this acute exposure to toxic fumes. In a survey of the region affected by the Exxon-Valdez oil spill, visual surveys completed in the hours following the spill did not find dead cetaceans (Harvey and Dahlheim 1994). Of the 80 Dall's porpoises (*Phocoenoides dalli*) sighted over 72 hours of observations, only one whale appeared stressed with laboured breathing and with oil covering the dorsal part of its body (Harvey and Dahlheim 1994).

An elevated number of cetacean mortalities have been recorded in the northern Gulf of Mexico beginning a few months prior to the Deepwater Horizon oil spill and extending into the present (as of December 2014) (NOAA 2014b). The U.S. National Oceanic and Atmospheric Administration (NOAA) declared the elevated cetacean mortalities as an "Unusual Mortality Event" (UME) according to the U.S. Marine Mammal Protection Act (NOAA 2014b). The possible cause(s) of death are being investigated and have yet to be identified (NOAA 2014b). NOAA (2014b) reports the number of stranded animals which includes those found dead but also those found alive but unable to return to its natural habitat or in need of medical attention. Stranding counts for cetaceans were: 114 prior to the Deepwater Horizon spill (February 1, 2010 to April 29, 2010), 121 during the initial response phase (April 30, 2010 to November 2, 2010) and, 1,060 after the initial response phase (November 3, 2010 to December 14, 2014) (NOAA 2014b). The majority of the dead animals were bottlenose dolphins (*Tursiops truncatus*) and mortalities were recorded for all year classes. It was also reported that the typical spring peak mortality of premature, neonate and stillborn dolphins was higher than normal in 2011 (NOAA 2014b).

It is also possible that mortalities immediately following oil spills are not observed but that toxic effects eventually lead to mortality. The possibility of delayed mortalities in killer whales (*Orcinus orca*) that frequent the waters of Prince William Sound, Alaska was investigated in the years following the Exxon-Valdez oil spill. Analysis of long-term annual census data of these killer whales collected from 1984 to 2005 showed that 33% and 41% of two distinct pods died within 18 months of the spill (Matkin et al. 2008). It was indicated that this mortality rate is greater than the average 2.5% per year typically observed in other pods. Matkin et al. (2008) argued that some of the whales may have died soon after the spill and that the remainder may have died later as a result of exposure by inhalation of fumes and the consumption of contaminated prey. Matkin et al. (2008) were not able to recover carcasses and perform necropsies to determine

exact cause of death. In another study, however, health assessments were completed on live bottlenose dolphins captured from oiled and non-oiled areas in the Gulf of Mexico following the Deepwater Horizon oil spill (Schwacke et al. 2014). This study provided rare insights into the pathologies of live cetaceans exposed to oil. Schwacke et al. (2014) found that dolphins from oiled areas were more likely to show signs of toxic stress that included hypoadrenocorticism and lung disease characterized by alveolar interstitial syndrome, lung masses and pulmonary consolidation. The authors argued that the observed symptoms were consistent with hydrocarbon exposure and taken together, they estimated mortality was possible for the dolphins in poor condition.

3.4.5 Mechanism of action: Narcosis

Narcosis is generally the mode of action that leads to acute toxicity from oil exposure. Following an oil spill, low molecular weight hydrocarbon compounds are most often associated with mortality observed during the first few hours to days. These compounds include some alkanes, monoaromatics (benzene, toluene, ethylbenzene, xylenes; BTEX) and PAHs (2-ringed naphthalenes).

Narcosis is a general term that actually involves many possible biochemical reactions in the organism. In aquatic organisms, narcosis is reversible and is similar to over-anesthesia. It is caused by hydrophobic chemicals partitioning into cell membranes and nervous tissue resulting in the general disruption of central nervous system functions (Barron et al. 2004). Also, narcotic organic chemicals, such as individual components of oil, appear to be toxic in an additive way (NRC 2005). This useful characteristic has been exploited in predictive models, such as target lipid models, that have been developed to estimate the expected toxicological impacts of oil on aquatic biota (French-McCay 2004; Di Toro et al. 2007). Toxicity due to narcosis is directly related to the fraction of lipid-soluble hydrocarbons, and thus its capacity to bioconcentrate in biota. Published K_{ow} for individual constituents of oil are used in these models to predict their solubility. In target lipid models, mortality by narcosis is then determined when the sum of the individual constituents of oil attain species-specific threshold concentrations in the target lipid (NRC 2005). In general, with increasing log K_{ow}, toxicity increases however its solubility in water decreases and thus, becomes less bioavailable (Di Toro et al. 2007). However, this approach of assigning toxicity based on hydrophobicity has been suggested to be an oversimplification in assessments of acute toxicity. There is evidence that log K_{ow} alone is not always correlated with toxicity and that multiple toxic pathways are possible, suggesting these models underestimate toxicity of oil (Scott and Hodson 2008).

While narcosis typically occurs within hours following a spill, delayed mortality is also possible after exposure to oil. Exposure to contaminated water or sediments can cause sublethal effects in individuals which can lead to a decreased probability of survival and reproduction and lead to a delayed response in population recruitment. To study this possibility of delayed effects, Heintz et al. (2000) incubated pink salmon (*Oncorhynchus gorbuscha*) eggs in sediments contaminated with low concentrations of crude oil (low ppb range) for approximately 8 months until the fry emerged. Following exposure, fish were raised in captivity, tagged and released as juveniles along with an unexposed control group. Two years later, when mature fish returned to spawn at their natal river, survival of exposed fish was estimated to be 15% lower than the control group. This experiment demonstrated delayed mortality can be a significant component of total mortality resulting from exposure to oil (Heintz et al. 2000).

3.5 SUBLETHAL TOXICITY OF PETROLEUM OIL

3.5.1 Embryotoxicity and early-life development

Early-life stages (embryos and larvae) of fish are particularly sensitive to oil exposure. Several factors may contribute to this, such as the low mobility of embryos and larvae stages compared to adults and thus, their inability to avoid contaminated areas. In some species, eggs are deposited on sediments where they undergo part of their development. Developing embryos can show toxic response if the sediments have been previously contaminated or if contamination occurs after deposition by adults. Some species of fish have eggs that float at or near the surface of the water. Irie et al. (2011) found that toxic response was expressed differently in floating eggs compared to sinking eggs. It was suggested that these eggs were exposed to different fractions of crude oil depending on their location in the water-column. Eggs floating on or near the surface were exposed to more water insoluble fractions while sinking eggs were exposed to more water soluble fractions (Irie et al. 2011). Some research has also shown that embryo and larvae of fish are more susceptible to PAH exposure than juveniles and adults as a result of the enhanced bioaccumulation by the early-life stages, potentially because of lower metabolism and higher lipid content (Petersen and Kristensen 1998). It has also been demonstrated that sensitivity to oil toxicity varies considerably between early-life stages. In a series of experiments, chemically dispersed oil toxicity was studied in gametes, embryos and free-swimming embryos of Atlantic herring (*Clupea harengus*) (McIntosh et al. 2010). This study showed that sensitivity to exposure was highest immediately following fertilization, and decreased with embryo age. Meanwhile, sensitivity to oil exposure was shown to increase again when hatched as a free-swimming embryo (McIntosh et al. 2010).

Following oil spills into the environment, harmful effects in early-life stages of fish can potentially lead to population-level effects. For example, several authors have associated the Pacific herring population decline in Prince William Sound, Alaska to harmful effects on embryos caused by the 1989 Exxon-Valdez oil spill. Substantial damage to embryos may have occurred given the overlap in timing of the herring spawn and the oil spill (Carls et al. 2002). It was estimated that 25-32% of Pacific herring embryos may have been damaged by the oil exposure (Carls et al. 2002). Although the direct link between the oil spill and the coinciding population decline is not clear, some authors have concluded that impaired recruitment in 1989 contributed to population declines in the years following the Exxon-Valdez spill (Carls et al. 2002; Thorne et al. 2008). In another example, during the recent Gulf of Mexico spill in 2010, the timing and spatial extent of spawning of several large pelagic predatory fish species overlapped with the Deepwater Horizon spill (Rooker et al. 2013). Laboratory experiments on early-life stages of Altantic bluefin tuna (*Thunnus thynnus*), yellowfin tuna (*Thunnus albacares*) and greater amberjack (*Seriola dumerili*) showed harmful effects when fish were exposed to environmentally relevant concentrations of field collected oil (Incardona et al. 2014). These experiments showed that oil exposure causes harm to cardiac function and development, suggesting that this could lead to impacts in wild populations (Incardona et al. 2014).

Individual effects - Deformities

Deformities are a commonly observed effect of oil exposure in early-life stages of fish. In 1989, following the Exxon-Valdez spill, field collections of Pacific herring larvae showed an elevated proportion of larvae with skeletal defects, including reduced and abnormal jaw structures and abnormal otic capsules, compared to control fish from un-contaminated regions (Hose et al. 1996). Laboratory experiments have also shown relationships between hydrocarbon exposure and deformities in fish embryos and larvae (Billiard et al. 1999; Brinkworth et al. 2003; Colavecchia et al. 2006; Boudreau et al. 2009; Incardona et al. 2014). A consistent finding in several of these studies is the development of a collection of symptoms referred to as blue sac

disease (BSD). These are often associated with developmental abnormalities including reduced growth, fluid accumulation in the yolk sac (edemas), pericardial edemas, spinal abnormalities, hemorrhages and mortality (Billiard et al. 1999; Brinkworth et al. 2003; Colavecchia et al. 2006; Boudreau et al. 2009). In a recent study, embryos of pelagic predatory fishes (i.e., tunas and amberjacks) developed pericardial edemas, deformed fins, curvatures in the body axis and reduced growth of eyes when exposed to low concentrations of total PAHs in the range of 3.4-13.8 µg/L (Incardona et al. 2014).

Individual effects – Heart-related effects

Cardiotoxicity and deformities in early-life stages appears to be a common observation across several types of oils and across many marine and freshwater fish species suggesting that underlying mechanisms are highly conserved across species. Wu et al. (2012) exposed rainbow trout embryos to four weathered crude oils that varied in classification from light to medium. Their results showed that characteristics consistent with blue sac disease were similar between oil types when compared on the basis of PAHs concentrations dissolved in water (Wu et al. 2012). Incardona et al. (2013) showed that there were largely indistinguishable differences in cardiotoxicity, morphological abnormalities and patterns of cytochrome P450 induction when zebrafish (*Danio rerio*) were exposed to Alaska North Slope crude oil (ANSCO) compared to crude oil from the Deepwater Horizon oil spill. Exposure to natural oil sands has shown similar patterns in cardiotoxic responses and developmental abnormalities in embryos and larvae of white suckers (*Catostomus commersoni*) and fathead minnows (*Pimephales promelas*) (Colavecchia et al. 2004; Colavecchia et al. 2006).

Mode of action: Aryl hydrocarbon receptor-mediated toxicity

Many of the toxic responses that have been documented in early-life stages of fish have been associated with binding of oil components to aryl hydrocarbon receptors (AhRs). The toxic mechanism of chemical binding to the aryl hydrocarbon receptor is not fully understood but involves CYP1A protein induction. Binding of some PAHs to aryl receptors have been related to the ability of different groups of invertebrates and vertebrates to metabolize and excrete PAHs. Fish readily metabolize non-chlorinated PAHs by cytochrome P4501A (CYP1A) monoxygenases during phase I metabolism (Galgani and Payne 1991). Briefly, PAHs bind to AhR and form ligand-receptor complexes that subsequently interacts with response elements and activates transcription of downstream genes such as the *cyp1a* gene (Denison and Nagy 2003). This leads to the increased synthesis of CYP1A proteins that metabolise PAHs and generally transforms the parent compounds into more soluble and readily excreted compounds.

Toxicity mediated by binding of PAHs with AhR can occur as a result of several mechanisms. Toxicity can occur as a result of the battery of genes that are expressed following binding of PAHs to AhRs (Denison and Nagy 2003). In zebrafish, for example, hundreds of genes are involved in the development of tissues and organs in embryos and larval stages (Haffter et al. 1996). Binding of PAH to AhR can cause continuous or inappropriate expression of some of these genes and cause harm in fish particularly during developmental stages. Toxicity can also occur as a result of oxidative stress when reactive oxygen species are produced during metabolism of PAHs by CYP1A enzymes. Monooxygenation of AhR ligands by CYP1A proteins can uncouple electron transfer of CYP1A proteins and lead to oxygen reduction, resulting in the release of O_2^- and H_2O_2 (Schlezinger et al. 1999). Production of these reactive oxygen species has been shown to contribute to oxidative damage in organisms (reviewed in Di Giulio and Meyer 2008). Toxicity can also occur as a result of reactive metabolites that can form as a by-product of metabolism of PAHs. In general, metabolism of organic compounds takes place in the liver and generates reactive metabolites that can cause damage to macromolecules such as nucleic acids (i.e., DNA, RNA) and proteins (Stegeman and Lech 1991). Further metabolism of

these reactive metabolites, by phase II conjugative enzymes, can also cause biochemical reactions that can lead to cellular damage (Stegeman and Lech 1991). Cellular damage and genetic damage can occur and these have been linked to early-life developmental effects, such as embryo deformities (Colavecchia et al. 2007; Clark et al. 2010).

Also, several studies have shown that 3- to 5-ringed alkylated PAHs, in particular, cause toxicity to early-life stages of fish (Carls et al. 1999; Barron et al. 2004; Wu et al. 2012). Carls et al. (1999) demonstrated that these substituted PAHs can be toxic even at very low concentrations, at parts per billion levels. Carls et al. (1999) further argue that toxicity of crude oil to early-life stages of fish likely depends on the proportion of alkyl-PAHs in the composition of the crude oil. Alkylated PAHs are the dominant form of PAHs in oil and, through the process of weathering, become further enriched relative to un-substituted PAHs (Wang et al. 2003). Alkylated forms are sometimes more toxic than the un-substituted counterparts suggesting that specific mechanisms or other factors, such as increased binding affinity to receptors, could be related to their enhanced toxicity (Turcotte et al. 2011). Studies have demonstrated correlations between embryo abnormalities and 3- to 5-ring PAHs concentrations (Wu et al. 2012). Furthermore, studies using single-compound exposures, rather than the complex mixtures typical of crude oils, found that alkyl phenanthrenes such as retene cause increases in symptoms of blue sac disease of exposed embryos of fish (Scott et al. 2011; Turcotte et al. 2011; Fallahtafti et al. 2012).

Toxicity of alkylated PAHs can also be mediated by AhR and related to the formation of reactive metabolites by CYP1A enzymes (Hodson et al. 2007). The importance of these metabolites to toxicity in larval fish has been shown by experiments that have blocked their formation using CYP1A inhibitors. An experiment co-exposing larval rainbow trout to retene and a CYP1A inhibitor found that blocking retene metabolism and thus, the formation metabolites, also prevented toxicity (Hodson et al. 2007). In contrast, when larval trout were exposed to retene only, tissue analysis revealed the presence of retene metabolites but not parent retene further suggesting that toxicity was primarily related to retene metabolites or the process of metabolism (Hodson et al. 2007). It is possible that toxicity by metabolites occurs when these are further metabolised by CYP1A enzymes forming reactive by-products. The role of metabolism in alkyl-PAH toxicity was demonstrated for a hydroxylated alkyl-PAHs, i.e., 1-methylphenanthrene, in early-life stages of medaka (*Oryzias latipes*) (Fallahtafti et al. 2012). It was found that metabolism by CYP1A enzymes can form reactive ring and chain hydroxylated derivatives and that toxicity was likely related to further metabolism of the derivatives and the formation of para-quinones (Fallahtafti et al. 2012). To date, only a few specific metabolites of alkyl-PAHs have been identified and have been shown to cause toxicity in fish including mortality and symptoms of blue sac disease in early-life stages (Hodson et al. 2007; Turcotte et al. 2011; Fallahtafti et al. 2012). Another mechanism of alkyl-PAH toxicity has been shown to specifically occur in cardiac tissues of early-life stages of fish. In this case, toxicity of alkylated PAHs is mediated by AhR receptors but independently from CYP1A enzymes. This mechanism was studied by exposing embryo-larvae life stages of zebrafish to retene (Scott et al. 2011). In this study, a gene knockdown approach using morpholino oligonucleotides showed that cardiovascular toxicity implicated binding to AhR2, an aryl hyrdrocarbon receptor isoform, but did not require activation of CYP1A enzymes. In particular, retene exposure to embryos of zebrafish affected the amounts of cardiac jelly deposited in the atrium. Cardiac jelly is likely important for maintaining elasticity of the developing heart of fish. Scott et al. (2011) suggested that a possible mode of action of toxicity is that binding of retene to AhR2 caused disruption of normal gene expression that is involved in the cardiac jelly secretion into the atrium.

Mode of action: Cardiotoxicity

Another mode of action related to cardiotoxicity in fish has been shown to be related to the disruption of heart conduction and to the activation of the aryl hydrocarbon receptor in cardiac tissues. Three-ringed PAHs (e.g., fluorenes, dibenzothiophenes, phenanthrene) in particular can cause direct toxicity to developing heart of fish embryos (Incardona et al. 2004; Brette et al. 2014). The underlying mechanism causing this disruption in heart conduction was related to blockage of potassium and calcium ion channels in cardiomyocytes (Brette et al. 2014). This caused disruption in normal functioning of electrical excitation and contraction coupling in cardiac tissues and led to increases in action potential duration, a predictor of lethal cardiac arrhythmia (Brette et al. 2014). Importantly, this disruption of the heart conduction and heartbeat has been found to precede morphological deformities of the heart and of other body tissues such as pericardial and yolk-sac edemas, craniofacial malformations, body axis curvatures and fin shape defects (Incardona et al. 2004; Incardona et al. 2014). It is also possible that these sublethal effects could lead to decreased growth and survival of juveniles.

Photo-enhanced toxicity

Solar radiation can also enhance the toxicity of hydrocarbons. Under some circumstances, this mechanism of toxicity can be significant as laboratory studies have shown that crude oil toxicity to fish can increase 1.5 to 1000-fold when exposed to full spectrum lighting compared to fluorescent lighting (Pelletier et al. 1997; Calfee et al. 1999; Barron et al. 2003). In general, two aspects of photo-enhanced toxicity can be considered: photo-oxidation and photo-toxicity. Photo-oxidation can be part of the weathering process, and occurs when solar radiation produces soluble oxidized compounds that contribute to the toxicity of weathered oil. Photo-toxicity does not involve oxidation but occurs within the organism after uptake of PAH (Lee 2003).

Photo-oxidized oil can produce several potentially toxic compounds such as hydroperoxides, phenols, carboxylic acids and ketones (reviewed in Lee 2003). Photo-toxicity includes the formation of free-radicals when sunlight reacts with PAHs. Some PAHs and alkyl-PAHs can absorb energy from UV radiation resulting in excited-state molecules that can produce reactive oxygen species or chemical modifications to PAH (Arfsten et al. 1996). It has been suggested that embryo and larva life stages of aquatic organisms are especially at risk because many are translucent to UV and occupy the photic zone of the water-column (Barron and Ka'aihue 2001).

3.5.2 Endocrine disruption effects on reproductive physiology

Individual / population-level effects

There is evidence that some PAHs can have endocrine disrupting properties that interfere with hormone-regulated processes such as reproductive physiology. However, following a hydrocarbon spill, the potential population-level impacts of hydrocarbon as an endocrine disruptor is not well understood. It may depend on the proportion of the population exposed. In a study following the Exxon-Valdez oil spill, plasma estradiol levels were measured in Dolly Varden trout (*Salvelinus malma*) collected in regions affected by oil contamination (Sol et al. 2000). In the year of the spill, it was estimated that 20% of the Dolly Varden trout population showed depressed 17β-estradiol compared to approximately 10% the following year (Sol et al. 2000). It was suggested that this might have had some negative impacts on the reproductive capacity of the population.

Several studies have shown that exposure to low molecular weight PAHs can cause disruption in reproductive development in fish such as delays in sexual maturation and reduced gonadal growth (Nicolas 1999). For example, exposure of Atlantic cod (*Gadus morua*) to low concentrations of water-accommodated fractions of crude oil (mean of 15-49 ppb total

hydrocarbon concentrations) for a period of 38-92 days caused disruption in gonadal growth and delayed spermatogenesis in male fish (Khan 2012). The author suggested that chronic exposure of PAHs during induction of gonadal development likely disrupted the release of reproductive hormones (Khan 2012). As these results suggest, exposure duration may be an important factor for causing endocrine disruption in fish. In a short-term (3-day) exposure of crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) to crude oil, there were no significant effects on plasma estradiol or testosterone concentrations, gonadosomatic indices, or histopathological organization of gonad tissues (Pollino and Holdway 2002).

Tissue-level: sex steroids

Endocrine disrupting chemicals can alter concentrations of sex hormones in plasma. In particular, an observed effect of PAHs on plasma hormones is the reduction of concentrations of 17β-estradiol (Nicolas 1999). Estradiol regulates the synthesis of vitellogenin which is a protein required in oocyte maturation and yolk incorporation, i.e., vitellogenesis (Goksøyr 2006). As a result, both plasma estradiol and plasma vitellogenin are often measured in studies of endocrine disruption in fish. PAHs such as phenanthrene, chrysene and naphthalene have been shown to cause a reduction in plasma 17β-estradiol concentrations in fish (Monteiro et al. 2000; Tintos et al. 2007). Reduced concentrations in plasma estradiol have also been found in fish collected from sites with contaminated sediments with aromatic hydrocarbons (Johnson et al. 1988). In a study in Puget Sound, Washington, female English sole (*Parophrys vetulus*) collected from contaminated sites had depressed levels of plasma estradiol and showed higher incidence of inhibited ovarian development compared to control fish (Johnson et al. 1988). This study suggests that depressed levels of sex hormones as a result of PAH exposure, could in some cases, lead to reduced spawning success in fish.

Hydrocarbons can also disrupt the endocrine system in aquatic invertebrates. In particular, filter-feeding bivalves have been studied because of their tendency to bioaccumulate organic compounds. Flow-through exposure to sublethal concentrations of crude oil caused increases in levels of estradiol in non-gonadal tissues (i.e., gills and mantle) of the mussel, *Mytilus edulis* (Lavado et al. 2006). In this experiment, estradiol sulfation also increased in digestion glands when exposed to oil, potentially reducing estradiol affinity for estrogen receptors (Lavado et al. 2006). In a another study, soft-shell clams that were fed pyrene-contaminated algae showed delayed gametogenesis in both males and females (Frouin et al. 2007). Here, Frouin et al. (2007) hypothesized that the delay in sexual maturation may have been related to alteration in steroid synthesis (e.g., possible interference in transformation of testosterone to estrogen) although this was not measured in their study.

In addition, other compounds such as surfactants can produce endocrine disruption in fish. In offshore oil production, surfactants such as alkylphenol ethoxylates used in pumping and drilling fluids can degrade into products that are known endocrine disruptors, principally alkylphenols nonylphenol and octylphenol (Meier et al. 2001). These alkylphenols have been shown to cause endocrine alteration in fish (Martin-Skilton et al. 2006). In laboratory experiments, turbot (*Scophthalmus maximus*) exposed to alkylphenols showed altered enzyme activities that are associated with the synthesis and metabolism of steroids along with changes in concentrations of testosterone and estradiol in plasma (Martin-Skilton et al. 2006). In contrast, the findings of this experiment indicated less measurable impact on Atlantic cod, suggesting species-level differences in response to these compounds. Similarly, several chemical dispersants used in countermeasure strategies following oil spills also contain the parent compound, nonylphenol ethoxylate. A study investigating the endocrine activity of 8 dispersants showed mild increases in estrogen receptor agonism with some dispersants but that Corexit 9500, which does not contain nonylphenol ethoxylates, showed no endocrine disrupting properties (Judson et al. 2010).

Mode of action of hormone disruption - molecular-level

There is some evidence that hydrocarbons can act as hormone mimics and bind to estrogen receptors (Tollefsen et al. 2006). This can cause activation (hormone agonism) or inhibition (hormone antagonism) of these receptors and disrupt the regulation of genes involved in reproduction and sexual differentiation (Goksøyr 2006). For hydrocarbons, binding to steroid receptors has been shown to involve mediation by aryl hydrocarbon receptors (Navas and Segner 2000). In-vitro experiments with rainbow trout liver cells demonstrated that CYP1A-inducing PAHs modulated estradiol regulated synthesis of vitellogenin (Navas and Segner 2000). In contrast, PAHs that do not induce the CYP1A enzyme system, such as anthracene, did not lead to antiestrogenic activity in the liver cells (Navas and Segner 2000). This suggests that metabolic transformation of PAHs may be needed to produce anti-estrogenic response (Nicolas 1999). Some PAH metabolites may compete against estradiol for estrogen receptors which could then lead to inhibition of synthesis of vitellogenin (Navas and Segner 2000). Another possible mechanism of action of endocrine disruption in fish involves interference with transport of natural hormones in plasma (Goksøyr 2006). PAHs have been shown to bind to sex steroid-binding proteins (Tollefsen et al. 2006; Tollefsen et al. 2011). These proteins play a role in circulating steroids in the plasma and binding to PAH could interfere with hormonal-regulated processes. In an exposure study with Atlantic cod, low concentrations of a crude oil mixture caused up-regulation of the total binding capacity of sex steroid-binding proteins for estradiol in female fish (Tollefsen et al. 2011). Tollefsen et al. (2011) suggested this increase in binding capacity may lead to an overall decrease in free and bioavailable estradiol in the plasma and cause disruption in hormone-regulated processes.

3.5.3 Physiological impairment

Petroleum hydrocarbons can also impact fish by disrupting the normal function of gills. Rainbow trout exposed to an oil–water emulsion showed impairment of osmoregulation under both hypo-osmotic and hyper-osmotic conditions (Engelhardt et al. 1981). Experiments showed that osmoregulatory disruption was from direct effects on the gills rather than from systemic effects (Engelhardt et al. 1981). These findings are consistent with other studies reporting impacts of crude oil and fish gills. Some of the morphological changes to gills following exposure to crude oil have included: swelling of epithelial cells, epithelial lifting, hyperplasia, aneurisms, proliferation of mucocytes, and lamellar fusions (Negreiros et al. 2011). These structural alterations of the gills are expected to affect the regulation of ions and the normal function of other physiological function such as gas-exchange (Negreiros et al. 2011). This could result in hypoxemia and a decreased ability to survive in low oxygen environments.

Petroleum hydrocarbon can also affect normal metabolic function and disrupt growth of fish. Experiments using mesocosms with oil-contaminated water and sediments caused reduced growth in flatfish for months after exposure (Claireaux et al. 2004). In this experiment, cellular ATP and ADP levels were depleted in fish and were correlated to structural changes in gill epithelium and decreased contractility of heart muscle. Additional studies showed that exposure to oil disrupts active metabolic rates more than basal metabolic rates leading to a net decrease in metabolic scope (Davoodi and Claireaux 2007).

Oil has also been shown to affect fish swimming abilities. Studies show that petroleum hydrocarbons can lead to sublethal effects to heart function and morphology (Incardona et al. 2014; Brette et al. 2014). The mechanism of action of cardiotoxicity in fish was discussed in Section 3.5.1. These effects to heart function and morphology could potentially affect survival of fast-swimming fish if abilities to attain elevated speeds required for feeding activities and for predator avoidance is compromised.

There is also evidence that other aspects of fish swimming performance can be altered when exposed to oil, potentially as a result of impaired energy budget or respiration. It was observed that small concentrations of the water-accommodated fraction of oil caused reduced swimming abilities of Pacific herring, and impaired their ability to recover from bursts of swimming (Kennedy and Farrell 2006). The observed effects increased in severity with increasing concentrations of oil and increasing duration of exposure. In a similar study, Coho salmon (*Oncorhynchus kisutch*) exposed to the water-accommodated fraction of oil exhibited decreased swimming performance even at low concentrations of oil (Thomas and Rice 1987). These observed impacts on swimming performance can be considered an integrative measure of fitness for aquatic animals as it is directly important for success in foraging and for avoidance of predators (Webb 1986).

3.5.4 Genotoxicity

Individual / population level effects

Exposure to hydrocarbons can damage genetic material (genotoxicity). Genetic damage can occur if exposure to genotoxic chemicals is at levels that exceed the rate of repair. In general, the impacts of genotoxins on organisms can include gamete loss, decrease in reproductive success, abnormal development, cancer, mutations that lead to embryo mortality and changes in genetic diversity (Würgler and Kramers 1992). For human health, the US EPA (1999) has listed probable PAH carcinogens as: benzo(*a*)pyrene, benz(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, dibenz(*a,h*)anthracene, and indeno(*1,2,3-cd*)pyrene. For aquatic biota, long-term exposure to these PAHs and to alkylated PAHs is also a potential concern and can cause genotoxicity. For example, benzo(*a*)pyrene has been recognized as a PAH causing genetic damage in fish (Metcalfe 1988).

Genotoxic impacts of environmental exposure to PAHs to individuals and populations are not fully understood. As reviewed in Diekmann et al. (2004), some researchers have suggested that the implications to populations may be minimal due to mechanisms such as selection and reproductive surplus. However, research following the Exxon Valdez oil spill showed that genetic damage occurred in Pacific Herring early-life stages (Hose et al. 1996; Hose and Brown 1998; Carls et al. 1999). In these studies, it was shown that herring larvae that hatched from eggs exposed to crude oil had elevated probability of genetic damage, which the authors argued could potentially have contributed to decreases in survival.

At the population level, it is also possible that genotoxicants can cause transgenerational response in fish. White et al. (1999) studied fathead minnows and found that exposure of parents to low concentrations of benzo(*a*)pyrene caused decreased survival in the next generation. In their study however, White et al. (1999) did not measure genotoxic endpoints and thus the response measured in the fish could not be directly linked to genetic damage. In another study, genotoxicity endpoints and reproductive aspects were studied in zebrafish (Diekmann et al. 2004). Adult fish were exposed to a model genotoxicant, 4-nitroquinoline-1-oxide, and its impacts were measured over the course of a full life-cycle. In this study, they found that genetic damage in adults led to a decrease in egg production which they suggest could lead to declines or disappearance of a population under some environmental conditions (Diekmann et al. 2004).

Tissue-level effects - neoplasia

Genetic damage can also be manifested as neoplasms, i.e., tumours. Some forms of neoplasia can develop into cancers. In fish, liver neoplasia has been incorporated into some monitoring studies as an indicator of fish health (Rafferty et al. 2009). In general, several field studies have

shown that fish inhabiting areas with contaminated sediments show greater frequencies of liver and skin tumours compared to reference systems (Reichert et al. 1998; Myers et al. 2003).

A few observational field studies have provided evidence that long-term exposure to PAH-contaminated sediments is correlated with the formation of neoplasia in fish. In general, most of these studies show a positive association between sediment PAH concentrations and prevalence of liver tumours. These include studies of brown bullhead (*Ictalurus nebulosus*) in tributaries of the Great Lakes (Baumann et al., 1991), English sole in Puget Sound, Washington (Reichert et al. 1998) and mummichog (*Fundulus heteroclitus*) in Elizabeth River, Virginia (Vogelbein and Unger 2006). Benthic fish species are more likely to be affected as a result of more frequent contact with contaminated sediments in comparison to pelagic species. There are also some studies that do not show a significant correlation between PAHs and liver neoplasia in fish (e.g., Pinkney et al. 2011). Other factors can contribute to the formation of tumours in fish (Pinkney et al. 2011). For example, some viruses have been shown to cause certain types of tumours in fish (Getchell et al. 1998). Also, areas with high rates of neoplasia in fish are often highly urbanised and industrial and presence of other stressors may contribute to the development of neoplasia. Few laboratory-based studies exist on the linkage between PAHs and neoplastic formation in fish (Vogelbein and Unger 2006). In one laboratory study, Metcalfe et al. (1988) showed that injecting extracts of PAH-contaminated sediments into rainbow trout can induce hepatocellular carcinomas.

Cause-and-effect studies have included the use of biomarker measures such as PAH metabolites in bile and the formation of DNA adducts. DNA adducts can form when DNA covalently binds to reactive PAH metabolites (Muñoz and Albores 2011). In some studies, these biomarkers of PAH exposures have been found to correlate with sediment PAH concentrations and frequency of liver neoplasia (Reichert et al. 1998). However, some studies have also reported a lack of relationship between these biomarkers and the formation of neoplasia in fish (Pinkney 2011).

DNA and chromosome level effects

Genotoxicants can cause DNA damage and/or chromosomal alteration. Bioassay tests have been used to study genetic damage following oil spills. An example of a common test is the micronuclei test which involves the detection of abnormal formations of a cell nucleus caused by damage to the centromere or defects in cytokinesis and is often used as an index of chromosomal damage (Barsiene et al. 2006). Genetic damage can also be manifested as a decreased ability of cells to successfully divide. Endpoints include a decrease in mitotic rate and an increase in mitotic aberrations. A commonly used test is the anaphase aberration test, a measurement of abnormal chromosome division, typically used on rapidly developing embryo-larval fish to ensure sufficient mitotic cells for analysis (Hose and Brown 1998). Another approach used to detect genotoxicity is to measure DNA single strand breaks using alkaline elution assays (Kohn 1991).

An example of genetic damage following exposure to oil is given in a study by Barsiene et al. (2006). In this study, researchers used micronuclei tests to study the potential of crude oil to cause genotoxicity. In their study, turbot and Atlantic cod were exposed to 0.5 ppm of physically dispersed crude oil for three weeks and showed significant increases in micronuclei frequency in erythrocytes and increases in other nuclear abnormalities in both fish species.

DNA and chromosomal-level damage has also been used in field genotoxicity assessments following accidental oil spills in the environment. Following the Exxon-Valdez oil spill of 1989, experiments showed that exposure to crude oil caused genetic damage in post-hatch Pacific herring amongst other sublethal effects such as deformities (Hose et al. 1996). Specifically, researchers collected newly-hatched larvae as well as incubated eggs from oil-affected areas

and found that anaphase aberration rates in larvae were significantly elevated compared to those from reference sites (Hose and Brown, 1998). In another example, research was conducted to assess the short-term (i.e., 4 months post-spill) and long-term (i.e., 7 and 10 years post-spill) genotoxic effects of the 1991 Haven tanker oil spill off the coast of Italy. Bolognesi et al. (2006) used indicators of DNA damage (i.e., DNA alkaline elution assays) and of chromosomal damage (i.e., micronuclei test) to measure possible genotoxicity on bivalves and fish near the sunken wreck of the Haven at 85 meters of depth. DNA damage was not detected in aquatic biota during the various phases of the monitoring program while it was possible to detect chromosomal damage in bivalves and in one of the fish species studied (*Mullus barbatus*) several years after the accident (Bolognesi et al 2006). The authors argued that after several years of the Haven accident, a residual presence of genotoxic compounds continued to cause harm to biota living near the wreck site.

Mode of action of DNA damage - biochemical-level

The mechanism of DNA damage has been related to the ability of some organisms to metabolise PAHs. Metabolism of PAH was discussed in the context of the sublethal effects of PAHs on the early-life stages of fish (see Section 3.5.1). PAH metabolism in fish increases solubility of the metabolites and facilitates excretion. It is also possible however, that genotoxic metabolites are formed during PAH metabolism. Phase I metabolism of PAHs involving the cytochrome P450 enzyme can form hydroxylated metabolites that can damage DNA by forming adducts or induce oxidative stress that can provoke mutations (Muñoz and Albores 2011). DNA adducts are formed when some reactive PAH metabolites, in particular benzo(*a*)pyrene metabolites, covalently bind to DNA (Muñoz and Albores 2011). A measure of DNA adduct formation is sometimes used as a biomarker of genotoxicity in aquatic biota exposed to PAHs (Harvey et al. 1999; Holth et al. 2009; Jung et al. 2011). Oxidative stress can also occur in some cases where PAH metabolites enter redox cycles and produce reactive oxygen species which can be converted to free radicals and react with DNA (Muñoz and Albores 2011). Overall, genotoxicity can occur in an organism when a substance is present at levels that cause rates of DNA damage greater than rates of DNA repair in the cell.

DNA adduct formation has been measured in aquatic biota in studies following exposure to PAHs. In the laboratory, Holth et al. (2009) exposed Atlantic cod to mixtures of PAHs and alkylphenols simulating realistic concentrations of discharged produced waters near offshore oil platforms (Holth et al. 2009). They found that more than 16 weeks of chronic exposure was needed to induce significantly elevated levels of DNA adducts in liver. In contrast, pulse exposures did not produce DNA adducts in cod. In another study, DNA adduct formation was measured in specimens following an accidental oil spill (Harvey et al. 1999). Following the 1996 Sea Empress oil spill in Milford Haven, Wales, UK, researchers collected marine invertebrates (i.e., a sponge and blue mussels) and teleost fishes (i.e., *Lipophrys pholis*, *Pleuronectes platessa* and *Limanda limanda*) in contaminated and reference sites. In samples collected from contaminated sites, DNA adducts were detected in the fish species.

3.5.5 Effects on immune system and resistance to disease

Immunotoxicity responses due to oil and PAH exposure have been shown across species of invertebrates, fishes, birds and mammals. The immunotoxic effects of petroleum are generally correlated with PAH components of oil and most studies have investigated the compounds 7,12-dimethylbenz(*a*)anthracene, benzo(*a*)pyrene and 3-methylcholanthrene (Reynaud and Deschaux 2006). Common immunotoxic responses to oil and PAH exposure across species are immunosuppression, inflammation, hemolytic anemia, decreased leukocytes and phagocytes, and impaired phagocytosis (reviewed in Reynaud and Deschaux 2006; Barron 2012).

Although it is generally known that PAHs can cause immunotoxic response in fish, this is not typically monitored or assessed following an oil spill. Following the Exxon-Valdez oil spill, Pacific herring received considerable research attention given its importance in the food-web. Adult herring captured in 1989, the year of the spill, showed greater incidence of liver damage in regions affected by oil compared to control regions (Marty et al. 1999). Some authors have suggested that this liver damage may have resulted from increased susceptibility to viral infections in fish that were exposed to crude oil (Marty et al. 1999; Carls et al. 2002).

Several types of immunity responses to PAHs are possible. In fish, specific immunity responses to PAHs involve cell- and humoral-mediated responses. In fish, T-lymphocytes are the primary cell type involved in cell-mediated responses (Reynaud and Deschaux 2006). Exposure to PAHs to fish has led to both increases and decreases in the production of T-lymphocytes (Reynaud and Deschaux 2006). It is currently not known why contradictory responses occur but may be related to various factors including dose concentrations, compound tested and species-dependent response of the immune system.

Other non-specific immunotoxic responses to hydrocarbons are also possible in fish. External lesions of fish gills, skin and fins have been reported in environments that have been contaminated with hydrocarbons. Some authors have suggested that this may be related to opportunistic infections caused by immunosuppression due to exposure to PAHs (Seeley and Weeks-Perkins 1991; McNeill et al. 2012). This immunosuppression has often been associated with decreases in macrophage activity in fish, i.e., in phagocytosis and chemotaxis (Reynaud and Deschaux 2006). In the case of oil-sands tailings ponds, immunosuppression was also reported. For example, rainbow trout released into tailings ponds for a period of 21 days showed fin erosion, amongst other responses (McNeill et al. 2012). The authors suggested that this was an indicator of opportunistic infection and that it may have been caused by factors such as PAHs, naphthenic acids and pH conditions.

Immunotoxicity due to PAH exposure has also been studied in benthic invertebrates. Phagocytosis is the predominant mechanism of immunity in bivalves. Studies have noted that phagocytosis tends to decrease when bivalves are exposed to PAH (Grundy et al. 1996a; Grundy et al. 1996b; Frouin et al. 2007). It has been hypothesized that PAH are directly toxic to lysosomes (Grundy et al. 1996b). In bivalves, several exposure routes have been shown to produce immunotoxicity. Experiments exposing bivalves to PAHs via contaminated phytoplankton, oiled sediments and fine residues from combustion of fuels have caused reduction in activity and efficiency of phagocytosis (Frouin et al. 2007).

A few studies have also shown that exposure to PAHs can lead to compromised resistance to pathogens in fish. In a laboratory experiment, juvenile chinook salmon (*Oncorhynchus tshawytscha*) were administered sublethal doses of PAH. Exposed fish were found to have an increased susceptibility to a subsequent exposure to the marine bacterium pathogen, *Vibrio anguillarum* (Arkoosh and Casillas 1998). In contrast, in another experiment, juvenile chinook salmon fed food contaminated with a mixture of PAHs for 28 days did not show any change in susceptibility to another bacterial pathogen, *Listonella anguillarum*, compared to control fish (Palm et al. 2003). It is possible that susceptibility to pathogens depends on factors such as route of exposure and on specific pathogen.

The mechanism of effects of immunotoxicity of PAH is not well known in fish. In a review by Reynaud and Deschaux (2006), it was discussed that findings from studies on both mammalian and fish models suggest that immunotoxicity responses involve different possible mechanisms including metabolic activation of PAHs by cytochrome P450, binding to aryl-hydrocarbon receptor and intracellular calcium mobilization.

3.5.6 Effects on behaviour

Limited information suggests that some fish species may alter their behaviour to avoid oil. A few studies have shown that adult and juvenile Coho salmon avoid monocyclic aromatic hydrocarbons, likely using their olfactory system (Maynard and Weber 1981; Weber et al. 1981). In laboratory experiments, Rice (1973) found that pink salmon fry avoided Prudhoe Bay crude oil above a threshold concentration of 1.6 mg/L in seawater. It is possible that these changes in behaviour in response to the presence of oil could be harmful to salmon populations by disrupting fish movement and seasonal migration.

There are a few studies that suggest PAH exposure can result in behavioural disruption. In fish, exposure to waterborne and sediment-bound PAHs has been shown to cause lethargy (Gonçalves et al. 2008) and changes in other behavioural traits such as anxiety level (Vignet et al. 2014a). In a recent study, Vignet et al. (2014b) fed zebrafish with food spiked with oil mixtures (heavy fuel oil or light crude oil) from 5 days post-fertilization until reproducing adults (6 months old). In this experiment, quantitative behavioural tests on both larval and adult fish showed that dietary exposure to oil resulted in increased mobility, decreased exploration behaviour and signs of increased levels of anxiety. The mechanism of action is not currently known but Vignet et al. (2014b) suggested that several are possible including disruption of dopaminergic and serotonergic systems and disruption of the developing nervous system.

For marine mammals, studies have demonstrated varying results on the ability of species to detect and avoid oil-contaminated waters (Engelhardt 1983). In cetaceans, aerial surveys in offshore regions of Atlantic Canada were conducted between 1979 and 1982 to monitor the presence of individuals near small oil slicks (Sorensen et al. 1984). Observers noted some individuals swimming near surface oil but rarely within surface slicks (Sorensen et al. 1984). The possibility of avoidance of oil spills by cetaceans was also studied following the 2010 Deepwater Horizon spill in the Gulf of Mexico. Ackleh et al. (2012) analyzed acoustic recordings of sperm whales (*Physeter macrocephalus*) before and after the spill and suggested possible avoidance of the spill affected area. Although, the authors also noted that avoidance could have been a result of increased human activities during the spill response. In contrast, following the 1990 Mega Borg oil spill in the Gulf of Mexico, aerial surveys showed that bottlenose dolphins did not consistently avoid contact with surface oil (Smultea and Würsig 1995). Dolphins were sometimes observed swimming through surface oil characterized as sheens and slicks but avoided mousse oil (Smultea and Würsig 1995). In another study, killer whales were observed swimming through surface oil within 24 hours of the 1989 Exxon-Valdez oil spill in Prince William Sound, Alaska (Matkin et al. 2008).

Some experimental work also investigated abilities of cetaceans to avoid oiled waters. Under experimental conditions, bottlenose dolphins held in captivity showed variable ability to detect oil floating on surface waters (Geraci et al. 1983). In these experiments, dolphins showed abilities to detect some crude oils but had reduced ability to detect refined products such as gasoline and diesel (Geraci et al. 1983). Smith et al. (1983) also showed that captive bottlenose dolphins initially avoided oil slicks in a small enclosure but that they eventually came into contact with oil.

3.6 TOXICITY OF CHEMICAL DISPERSANTS AND CHEMICALLY DISPERSED OIL

3.6.1 Toxicity of chemical dispersants

Toxicity of chemical dispersants has changed considerably over the years. Early formulations were significantly more toxic. For example, the LC50 (96 h) values of dispersants used in the 1970s ranged from 5 to 50 mg/L for rainbow trout. In contrast, average LC50 (96 h) for dispersants available in the 2000s ranged from 200 to 500 mg/L (Fingas 2011b). In a recent study, the acute toxicity of eight chemical dispersants, including Corexit 9500A, the dispersant

primarily used during the 2010 Deepwater Horizon oil spill, were tested using a mysid shrimp species (*Americamysis bahia*) and silverside fish (*Menidia beryllina*) (Hemmer et al. 2011). In this study, all eight dispersants were found to be less toxic than chemically dispersed oil used for comparison. The LC50s for both species (48 h for *A. bahia* and 96 h for *M. beryllina*) for the chemical dispersants alone ranged from 2.9 to >5,600 µl/L while, for the dispersed oil mixture the LC50s ranged from 0.4–13 mg TPH/L (Hemmer et al. 2011). A full review of dispersant toxicity to aquatic biota and the use of chemical dispersants are not covered in this report but have been the subject of several reviews (Fingas 2011b; NRC 2005; Chapman et al. 2007).

3.6.2 Toxicity of chemically dispersed oil

A substantial amount of research has been conducted to examine if the application of chemical dispersants is more harmful to aquatic biota than untreated oil. The use of chemical dispersants, in combination with mechanical energy, favours the development of oil-surfactant micelles measuring <100 µm (NRC 2005). The movement of oil droplets from the surface into the water-column also initially increases PAH concentrations below the surface (Fingas 2011b). Studies in the laboratory have shown that for equal loading of oil, chemical dispersants can temporarily increase the exposure of pelagic organisms to oil components compared to treatments where oil is physically dispersed (Wolfe et al. 2001; Ramachandran et al. 2004; Couillard et al. 2005; Adams et al. 2014). In a laboratory experiment, rainbow trout were exposed to chemically dispersed oil (i.e., chemically enhanced water-accommodated fraction, or CEWAF) and physically dispersed oil alone (i.e., water-accommodated fraction, or WAF) (Ramachandran et al. 2004). This study showed that exposure to oil differed in treatments and that exposure to hydrocarbons, as measured by CYP1A induction, was increased in the CEWAF treatment.

The National Research Council (2005) found that many of the publications that concluded that chemically dispersed oil increased the risk of toxicity did not include actual measures of the oil components in water but rather inferred concentrations based on loading (nominal concentration) or based on dilution of a measured stock solution. In addition, studies have shown that experimental protocols and study design can affect results and interpretations of the toxicity of physically dispersed oil compared to chemically dispersed oil (Singer et al. 1998). Several authors have made recommendations to address problems with testing and result interpretations (see Section 3.2.1).

3.7 TOXICITY OF OIL SANDS PRODUCTS

There is currently a lack of research assessing the potential effects of diluted bitumen and synthetic crude oil (upgraded bitumen) on aquatic organisms. In contrast, work is ongoing for monitoring the environment near oil sand activities. In 2012, the Governments of Canada and Alberta implemented an environmental monitoring and research plan for the oil sands region which was designed to enhance existing monitoring activities. As part of this program, monitoring of aquatic biota (i.e., fish and invertebrates) health is ongoing upstream and downstream of oil sands developments and also, in mining development areas. Also, laboratory-based experiments on fish were conducted to test the toxicity of melted snow collected from sites near oil sands mining and upgrading facilities and from reference locations away from the influence of mining activities. Activity summaries and data are made available via a publically accessible web portal.

As with other oils, the primary component of concern in oil sands products are PAHs and particularly alkylated PAHs (Colavecchia et al. 2004). Several studies have investigated the toxicity of natural oil sands deposits on fish (Tetreault et al. 2003; Colavecchia et al. 2004; Colavecchia et al. 2006; Colavecchia et al. 2007). In the Athabasca oil sands region, these natural bitumen deposits can be found exposed in the banks of rivers where natural erosion

processes can allow leaching of these compounds. In the laboratory, these natural oil sands deposits were shown to cause toxicity in early-life stages of fish (Colavecchia et al. 2004; Colavecchia et al. 2006). In these experiments, fathead minnow and white sucker eggs and embryos were exposed to oil sands sediments. Compared to controls, the fish eggs and embryos showed increased mortality, reduced hatching success, delayed timing of hatching, abnormal embryo development, larval deformations and reduced size of larvae (Colavecchia et al. 2004; Colavecchia et al. 2006). Larval deformities included exposure-related increases in the prevalence of spinal defects, edemas (pericardial, yolk sac, and sub-epidermal), and hemorrhages (Colavecchia et al. 2004; Colavecchia et al. 2006). These toxicological responses were noted by the authors to be similar to that previously described for other weathered crude oils.

Studies of wild fish in the oil sands region found that exposure to natural bitumen caused sublethal biochemical and hormonal responses (Tétreault et al. 2003). Common species in the Athabasca watershed such as slimy sculpin (*Cottus cognatus*) and pearl dace (*Margariscus margarita*) showed reductions in steroid production and increases in EROD activity compared to fish in reference areas (Tétreault et al. 2003). For early-life stages of fish, it is suggested that these biochemical responses can be related to the observed deformities in embryos and larvae following exposure to oil sands affected waters (Colavecchia et al. 2007).

4 RESEARCH RECOMMENDATIONS

Research recommendations were developed based on the existing knowledge gaps on the biological effects of oil on aquatic organisms. This section was developed as a result of discussions with participants at a Canadian Science Advisory Secretariat (CSAS) workshop for identifying research requirements for the biological effects of oil on aquatic ecosystems (DFO 2015).

Oil Toxicity Research Recommendations

1. Toxicological studies on oil sands-related products such as natural bitumen, diluted bitumen, synthetic crude oil and bitumen blended with synthetic crude oil.
    a. Determine appropriate study species for standardised testing
    b. Conduct lethal and sublethal toxicity tests, early-life stages tests
    c. Compare the toxicity of dilbit, synbit, and syncrude relative to other products; if they are uniquely toxic, determine mechanisms of effect
    d. Establish a reference dilbit product for standardised toxicity testing using a standard suite of chemical analyses and effects endpoints
2. Assessment of fate and behaviour of diluted bitumen following a spill in aquatic environments
    a. Identify areas and habitats of greater risk of oil spill, and fate of oil within these systems
    b. Improve modeling capacity
    c. Developing clean-up methods appropriate to unique ecosystem characteristics
    d. Ecotoxicology of conventional and unconventional crude oil products in ice-covered Canadian waters
3. Assessment of potential consequences of a condensate spill to aquatic organisms
    a. Validation of existing acute toxicity models and/or development of new models as required
    b. Determine exposure potential - fate and behaviour in the aquatic environment
4. Improved ecological relevance of oil and gas toxicity studies
    a. Establish cause and effect in field-based studies
    b. Validate laboratory studies using field-based approaches
    c. Identify susceptible life-history traits and relate to oil fate characteristics
    d. Extend research beyond individual-effects to population, community, ecosystems
    e. Develop the capacity to assess biological effects during and after spill events, especially recovery times
5. Demonstrate mechanisms of chronic toxicity in support of improved predictive models
    a. Identify characteristic exposure responses
    b. Identify unique biomarkers of exposure to bitumen
    c. Conduct pharmacokinetic studies
    d. Identify unique chemistry profiles of oil products, including geochemical markers
    e. Determine the role of oil droplets (residual oil) in estimating toxicity
    f. Establish the relative importance of photo-toxicity after an actual oil spill.
6. Assessment of biological effects of chemically dispersed bitumen
7. Potential effects of metals in bitumen to benthic organisms if sinking occurs

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7 GLOSSARY

Acute exposure: short term exposure lasting hours to days. Time of exposure is relative to organism lifespan.

Acute toxicity: single exposure of a toxin that produces symptoms within a short period of time after exposure. Generally, its manifestation is mortality.

Bioaccumulation: the tendency of substances to accumulate in the body of exposed organisms over time or with age.

Bioassay: the measurement of a substance by the reaction that it produces in living tissue or organism.

Bioavailable: in a form that can be assimilated by a living organism, usually related to the physical or chemical form of a substance.

Biomarker: subcellular biochemical and molecular responses to some environmental change, e.g., ethoxyresorufin-*o*-deethylase activity (see EROD).

Bitumen: a heavy, viscous oil extracted from oil sands deposits that must be processed to convert it into a crude oil for use in refineries for the production of gasoline and other petroleum products. Processing can involve upgrading (see Upgrading).

BTEX (benzene, ethylbenzene, toluene and xylene): a group of volatile organic compounds with similar physical and chemical properties, and typically, toxic to organisms.

Condensate: a mixture of pentanes and other hydrocarbons. Condensate is sometimes used as a diluent and mixed with bitumen to decrease overall density and viscosity for transport.

Conventional crude oil: a mixture of pentanes and heavier hydrocarbons extracted at a well from an underground reservoir. It is liquid at the conditions under which its volumes are measured. Conventional crude oil does not include raw gas, condensate, or bitumen.

Chronic exposure: long term exposure typically lasting weeks or months to years. Time of exposure is relative to organism lifespan.

Chronic toxicity: the potential long term effects which could result from exposure to small amounts of a toxin over time. Does not usually include mortality but resulting damage to health and other effects can lead to death.

CYP1A enzymes: the gene family of cytochrome P450 enzymes responsible for transformation of xenobiotic and endogenous substrates. Forms part of the enzyme system responsible for the transformation of non-polar organics to more polar, water soluble products.

Dispersants: mixtures of surfactants, solvents and other additives that are applied to oil slicks to reduce the oil-water interfacial tension thereby promoting the formation of small oil droplets with wind and wave energy. Small droplets disperse more readily into the water column and can be biodegraded by microorganisms.

Embryotoxicity: toxicity occurring to the developing embryo.

Endocrine disruption: interference with the production, action, or elimination of natural hormones responsible for the maintenance of homeostasis and the regulation of physiological processes.

Endpoint: a biological process used to quantify response from exposure to toxic substance. Measures are chosen by the experimenter.

EROD (ethoxyresorufin-*o*-deethylase): a catalytic measurement of cytochrome P450 induction commonly used as a biomarker of exposure to contaminants in fish.

Exposure: in toxicology, refers to the amount of a substance in an organism's immediate environment.

HMW hydrocarbons (high molecular weight hydrocarbons): classification of hydrocarbons based on weight of molecule. This includes polycyclic aromatic hydrocarbons (PAHs) with 4 or more benzene rings, some alkanes, resins and asphaltenes. See also LMW hydrocarbons.

Hydrocarbon: any organic compound composed solely of the elements hydrogen and carbon.

LC50: median lethal concentration, the concentration of a substance that will kill 50% of a standard population of organisms in a given time, typically a short time, e.g., the 96-hour bioassay.

LMW hydrocarbons (low molecular weight hydrocarbons): classification of hydrocarbons based on weight of molecule. This includes polycyclic aromatic hydrocarbons (PAHs) with 2 or 3 benzene rings, monoaromatics (see BTEX) and some alkanes. See also HMW hydrocarbons.

Metabolite: any product of metabolism.

Octanol-water partition coefficient (log K_{ow}): the distribution of a given substance between octanol and water, at equilibrium. Generally used as an indicator of the tendency of a substance to move from water to lipid and thus its tendency to move from the aqueous environment into biological membranes.

Oil sands: refers to a mixture of sand and other rock materials containing bitumen. In Canada, natural deposits of oil sands are found primarily in Alberta.

PAH (polycyclic aromatic hydrocarbon): hydrocarbons that contain more than one benzene ring, e.g., anthracene, benzo(a)pyrene.

Persistence: in toxicology, the tendency of a substance to remain essentially unchanged over time in the environment or in an organism.

Petroleum oil: liquids composed of mixtures of hydrocarbons and other compounds that include naturally occurring unprocessed crude oil; refined oil products derived from crude oil and blended oil products.

Refined petroleum products: end products in the refining process (e.g. gasoline, diesel).

Solubility: the ability of a substance to dissolve into a liquid.

Sublethal toxicity: changes in physiological processes, growth, reproduction, behavior, development, and other non-lethal endpoints resulting from exposure to a toxin. Generally, involves long term exposures.

Synthetic crude oil: a mixture of oil derived by upgrading bitumen (See Upgrading).

Toxicity: damage to a living organism caused by exposure to a chemical. See also, acute toxicity and sublethal toxicity.

Upgrading: the process that converts bitumen or heavy crude oil into a product with a lower density and viscosity. Some bitumen is upgraded to synthetic crude oil prior to transport while some bitumen is not upgraded but mixed with lighter diluents to decrease overall density and viscosity for transport.